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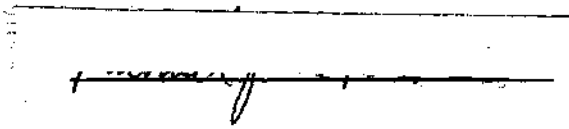

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THE EFFECTS OF COAGULATION UPON THE RESISTIVITY AND PERMITTIVITY OF HUMAN BLOOD PLASMA

A THESIS

Presented to

The Faculty of the Division of Graduate

Studies and Research

by

Michael Jesse Buckler

In Partial Fulfillment

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Master of Science in Electrical Engineering

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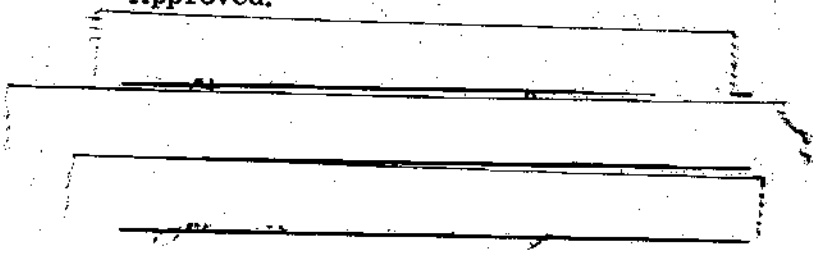
THE EFFECTS OF COAGULATION UPON THE RESISTIVITY AND PERMITTIVITY OF HUMAN BLOOD PLASMA

Approved:



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SUMMARY

The objective of this thesis was to measure and record the effects of coagulation upon the resistivity and permittivity of human blood plasma. In order to accomplish this objective it was necessary to design, build, and analyze an electronic measurement system capable of measuring and recording the effects of coagulation upon the resistivity and permittivity of human blood plasma. A survey of the experimental methods and results of previous investigations performed on electrical characteristics of whole blood and blood plasma during coagulation uncovered many inconsistencies among the previous works. Most authors did not completely document their probe configurations or methods of converting their resistance and capacitance data to values of resistivity and permittivity. None of the previous works from the survey stated the accuracy of their method of temperature control, or of the actual electrical measurements. Since the liquids used in this work were 75 per cent human blood plasma and 25 per cent 0.02 molar calcium chloride ($CaCl_2$), the results of this thesis are not directly comparable to other researcher's results on whole blood or blood plasma.

A probe using two stainless steel electrodes separated by a teflon spacer was used for the resistance and capacitance measurements. An analysis and calibration of the probe was accomplished so that the resistance and capacitance measurements could be converted into corresponding resistivity and permittivity data.

Separate measurements of resistivity and permittivity were made at 20 KHz using two different a.c. bridges for each human blood plasma-$CaCl_2$ sample tested. Tests were performed upon 300 different human blood plasma-$CaCl_2$ samples under carefully controlled and well documented experimental conditions. The procedure used in the treatment of the 300 samples was carefully documented. The effects of temperature on the resistivity and permittivity of the samples has been recorded, and compensation techniques were employed to minimize these effects.

The permittivity of each of the 300 samples tested increased during coagulation. The average percentage change in permittivity was +3.19 per cent. Of the 300 samples tested, 99.7 per cent had a permittivity increase between .06 and .50 pF/cm, with an average increase of .20 pF/cm (20 pF/m or a relative dielectric constant change of +2.26). The accuracy of the measurement system used to measure the changes in permittivity was $\pm$ .50 per cent. Thus, the average increase in permittivity was more than six times the accuracy of the bridge used to make the permittivity change measurements.

No uniform positive or negative trend was apparent from the resistivity changes of the 300 samples tested. The average resistivity change due to coagulation of the 300 human blood plasma-$CaCl_2$ samples was less than $\pm$ .50 per cent. No resistive variations greater than $\pm$ 3.6 per cent were evident during the 300 test runs.

The amount of correlation between the resistivity and permittivity changes of the 300 samples tested was .0155. Thus, there was no apparent correlation

between resistivity and permittivity changes of human blood plasma-$CaCl_2$ samples due to coagulation.

CHAPTER I

INTRODUCTION

The Problem

All of the many instruments designed to follow the coagulation of blood or plasma have certain deficiencies from the standpoint of a practical clinical laboratory device.[1] Many suffer from poorer accuracy than can be obtained by a well trained technician using the Lee White test.[2] Others are based on designs which are inherently too expensive for a routine laboratory device.[3]

Various attempts have been made to study the process of blood coagulation by determining changes occurring in its physical properties. Properties investigated in this regard include variations in viscosity,[4] elasticity,[5] light transmission,[6] and electrical conductivity.[7] Such investigations have usually shown a fairly quiescent period for the first 3-5 minutes from the onset of coagulation. Changes in factors concerned with the initial phases of coagulation can, thus, apparently pass undetected by the physical methods used to date. Therefore, it was decided to investigate other properties such as resistivity and permittivity of human blood plasma which may bear some relation to coagulation.

History

A review of the literature indicated that gross inconsistencies exist among what various authors have reported concerning changes in the electrical parameters

of blood plasma and whole blood during coagulation.

Rosenthal[7] experimented with human whole blood at 37°C in his tests. He reported resistance increases from 160 ohms before coagulation to 420 ohms after coagulation. His probe apparatus consisted of electrodes made of lightly platinized platinum plates 0.01 inch in thickness, 0.25 square centimeter in area, and .50 cm apart immersed in a 12 mm diameter Pyrex tube. He employed an impedance bridge operating at a frequency of 1000 Hz, with an applied input voltage of 0.25 volts rms. Rosenthal calibrated his measurement system using a standard solution of 0.1 molar KCl. Thus, he determined a conductivity cell constant which allowed for the conversion of his resistance measurements to values of specific resistance (or resistivity).

Graff[8] used human whole blood at 37°C for his studies. He reported resistance increases from 100 ohms before coagulation to 210 ohms after coagulation. His measurements were made using a 5 volt rms, 60 Hz input signal to an a.c. impedance bridge. Graff also reported a conversion factor which he identified as a conductivity cell constant for converting resistance values into resistivity data. He did not report what standardizing solution or probe configuration were used to obtain his conductivity cell constant.

Henstell[9] also used human whole blood at 37°C for his measurements. He reported that the resistance of human whole blood increases during coagulation. Typical initial and final values of resistance were respectively 198 and 265 ohms. Henstell used electrodes constructed of platinum wire (.02 inches thick) in the form of a circle with a horizontal cross-bar. The diameter of the

circle was .211 inches with an electrode separation of 1.5 mm. This probe configuration was inserted into pyrex glass test tubes (diameter of .450 inches) filled to a depth of 1.05 inches with blood. The instrument used to measure resistance was a Leeds and Northrup 'pre-micromax' a.c. galvanometer. Henstell did not report any calibration procedures or conversion methods to convert his resistance data to resistivity values.

Wilson[10] used whole rabbit blood for his studies. He reported increases of 5-19 per cent in the resistance, with typical initial and final values of 6413 to 7910 ohms, respectively. Wilson did not document his measurement system, probe configuration, or any method of converting his resistance data to resistivity values.

Ott[11] used human whole blood at 37°C for his tests. He reported erratic increases and decreases of resistance during coagulation and could not formulate any feasible conclusions about his results. Neither the method, nor apparatus used for obtaining his data, was documented.

Mungall[12] used a capacitance-resistance bridge at 100 KHz for the measurement of the dielectric properties of 60 human whole blood samples at 37°C. "The electrodes and leads were made of platinum." Mungall concluded that, "There was not sufficient data available to sort out the many variables which influence the measurement." No documentation was given on how to convert his erratic values of resistance and capacitance to values of resistivity and dielectric constant.

Frank[13] used human whole blood for his experiments. Freshly drawn blood was caught in small vessels, paraffined to retard coagulation, and kept at constant

body temperature until clotting was completed. The conductivity was determined at frequent intervals during the entire process. Electrodes of 2-3 square centimeters surface, heavily platinized, were used. They were separated between 1-2 cm, depending upon the amount of blood obtainable. Frank concluded that there was no appreciable change in conductivity during coagulation. No documentation was given on the measurement system.

Schwan measured the dielectric constant relative to air, and the specific resistance in ohm-centimeters of one sample of beef whole blood at 27°C, and 200 MHz.[14] He reported a dielectric constant of 67, and a specific resistance of 96 ohm-cm. Using an impedance model which he derived for the dielectric parameters of tissues, Schwan calculated a typical value of dielectric constant of beef blood to be 57, at 200 MHz. He attributes the theoretical inaccuracy to surface polarization, structural dispersion, and polar water dispersion.

Attempts to compare various results of the above cited works, proved to be extremely difficult, since most authors did not adequately document the experimental environment under which their results were obtained. None of the previous works have stated the accuracy of their method of temperature control, or of actual electrical measurements.

In conclusion, a survey of the experimental methods and results of previous investigations performed on electrical characteristics of blood and blood plasma during coagulation points up the lack of documentation and the inconsistencies in the previous works.

CHAPTER II

THESIS OBJECTIVE

The primary objective of this thesis was to measure and record the effects of coagulation upon the resistivity and permittivity of human blood plasma. In order to accomplish this objective, it was necessary to design, construct, and calibrate an electronic measurement system capable of electrically measuring and recording the resistivity and permittivity changes of human blood plasma samples due to coagulation. The plasma samples were treated with a $CaCl_2$ solution to cause clotting. The measured changes in the electrical parameters of human blood plasma-$CaCl_2$ mixtures could possibly be used to identify the clot point, disease formation phenomena, or other medical analyses.

A refined, balanced bridge instrumentation system was employed to determine the resistivity and permittivity changes of human blood plasma-$CaCl_2$ due to coagulation. A parallel plate probe was inserted into plasma samples. This probe was also immersed in other media to calibrate the system. Approximately 300 samples were tested to insure repeatability of the experiment, and to provide sufficient data for a statistical analysis of the results. A statistical analysis of the results was performed by calculating the mean value, standard deviation, and correlation coefficient of the 300 changes in resistivity and permittivity.

A secondary objective of the thesis was to perform these tests under well

documented experimental conditions. The effects of temperature variations, probe position, and probe materials were investigated to insure the repeatability of the measurements.

A review of the literature indicated that gross inconsistencies existed among what various authors had reported concerning the electrical parameter changes of blood plasma, and whole blood during coagulation. Thus, since the measurement system was calibrated for the probe configuration used in this thesis and since the laboratory experimental conditions were well documented, this research will serve as a solid foundation and building block for future work done in the area of electrical characteristics of human blood plasma-$CaCl_2$ solutions.

CHAPTER III

EXPERIMENTAL APPARATUS AND TECHNIQUES

Sample and Probe Model

The blood plasma and $CaCl_2$ coagulant mixture along with the probe were modelled as a one-port black box as shown in Figure 1. (The blood plasma and $CaCl_2$ mixture will be referred to henceforth as the plasma sample). According to circuit theory, any unknown linear, passive, one-port network can be represented as a series or parallel RLC circuit. Since the plasma sample and probe have a finite dc resistance,[15] the series model would fail at f=0. If it is assumed that the input frequency is different from the resonant frequency of the LC combination, either L or C will dominate, making the net reactance either positive or negative. Tests between 1 KHz and 20 KHz have consistently shown the reactive component to be negative, indicating the predominance of the capacitance.[16] Work done by Ferris with general electrolytes also showed this result.[17] Therefore, the plasma sample and probe were modelled as a parallel RC combination.

Probe

The probe shown in Figure 2 was used for the measurement of resistivity and permittivity. The probe parameters in air were measured on a Hewlett-Packard Model 4260A Universal Impedance Bridge. It was found that the probe capacitance was .43 pF. The probe resistance exceeded ten meg-ohms.

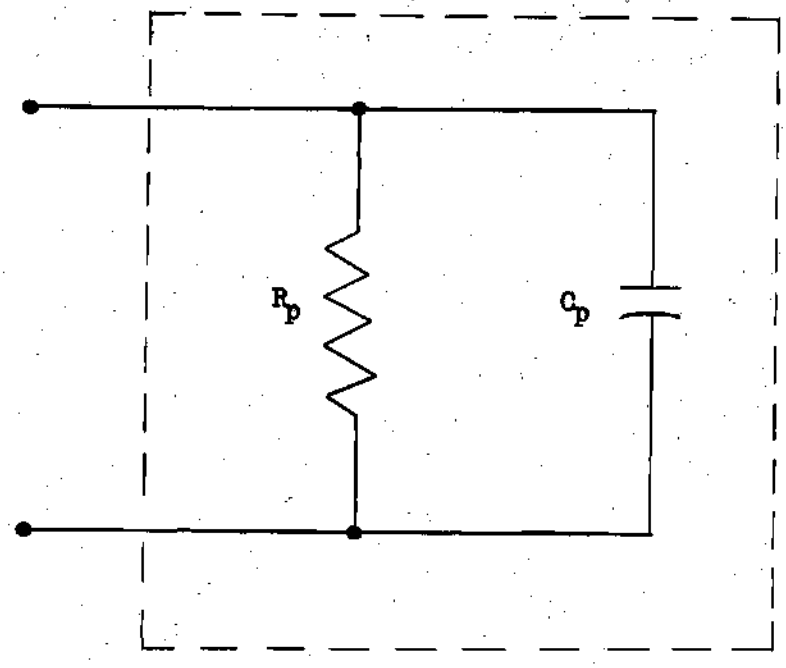
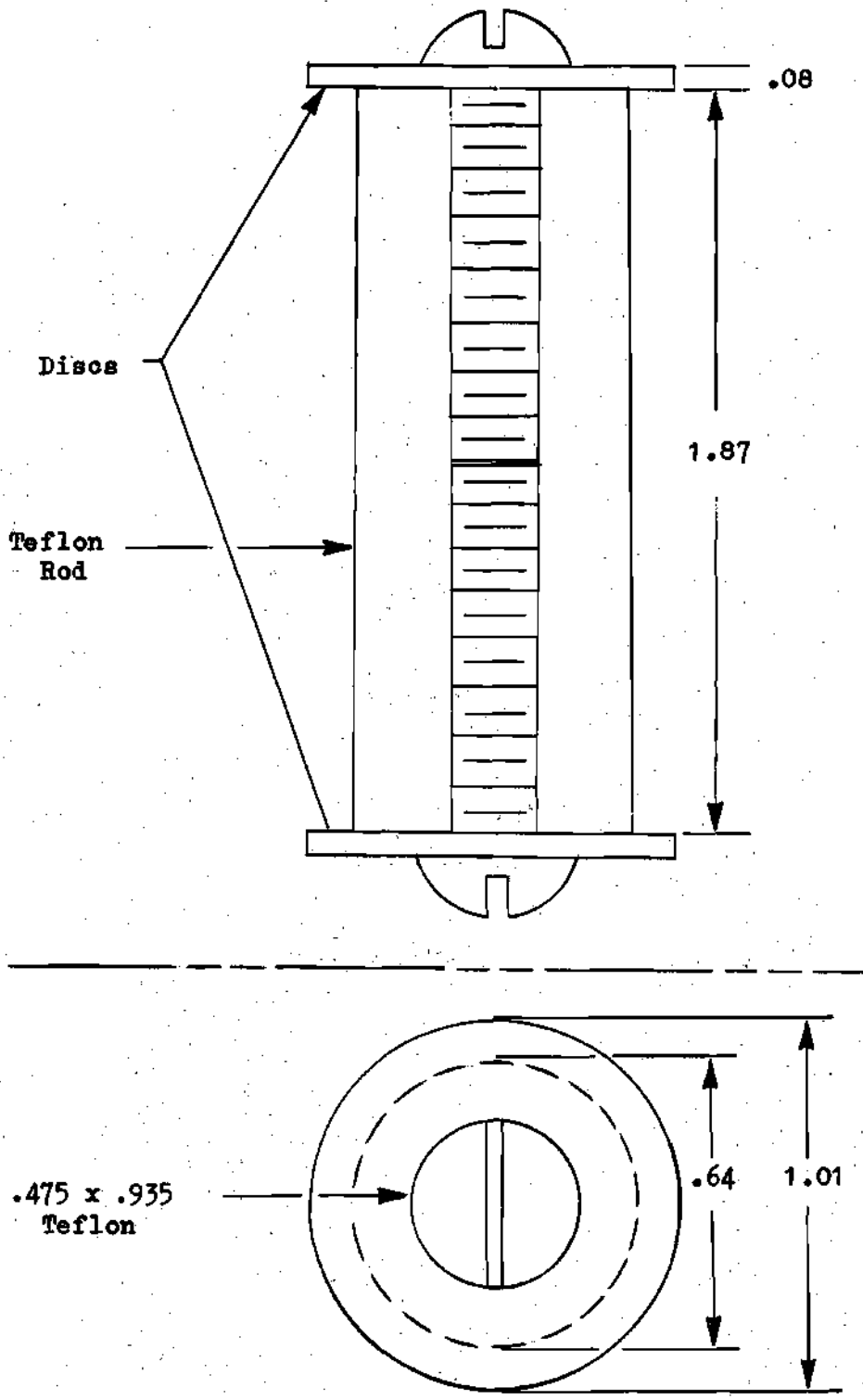


Figure 1. One-Port Plasma Sample And Probe Model.



(1) All dimensions in centimeters

(2) Area of plates .475 cm.^{2}

Figure 2. The Probe.

The probe was constructed using two 1.01 cm. stainless steel discs separated by a teflon 101 spacer. By using the basic definitions of resistance and capacitance for a straight rectangular or cylindrical length of material, the resistivity and permittivity can be expressed as:

$$\rho = R_p A/L \tag{1}$$

$$\epsilon = C_p L/A \tag{2}$$

where

ρ = resistivity of the dielectric medium between the plates, in ohm-cm.

ϵ = permittivity of the dielectric medium between the plates, in farads/cm.

R_p = equivalent resistance of the sample and probe, in ohms.

C_p = equivalent capacitance of the sample and probe, in farads.

L = separation distance of the plates in centimeters.

A = surface area of the plates in square centimeters.

Using the dimensions shown in Figure 2, then

$$\rho = .254\, R_p \tag{3}$$

$$\epsilon = 3.94\, C_p \tag{4}$$

The diameter of the circular probe plates is 1.01 cm., and the inside diameter of the test tubes used for these tests is nominally 1.02 cm. The

difference in the diameter of the probe plates and the inside diameter of the test tubes is to allow for a wire from the bottom plate of the probe to pass between the probe plates and the inside wall of the test tube, out to the measurement system. It was assumed that there was essentially a straight column of plasma between the probe plates. Therefore, the probe arrangement is similar to setups used to measure the resistivity and permittivity of standard lengths of material. Thus Equations (3) and (4) will be used as a first order approximation to determine the resistivity and permittivity of plasma samples. The verification of this approximation and the probe calibration procedure will be discussed in Chapter III.

Measurement System

A block diagram of the electronic measurement system which was designed to measure the resistance and reactance of the plasma samples is shown in Figure 3 (with the exception of the Audio Oscillator, Strip Chart Recorder, and the Bridge System this measurement system represents an original design carried out by the author to measure the resistivity and permittivity changes). The audio oscillator supplies a 20 kHz, sinusoidal, 100 mV peak-to-peak signal to the system. This signal passes through a 20 kHz notch filter to suppress all spurious signals present on the incoming 20 KHz signal. The signal is then split into two parts. One signal is fed to the "Bridge System." The functional block in Figure 3 called "Bridge System" is the bridge circuitry used by Neale C. Hightower in his research for his Master's Thesis (see Figure 4). The bridge in Figure 5a (denoted as Bridge I), was used to measure the resistivity of the plasma samples. The bridge in Figure 5b (denoted as Bridge II), was used to measure the permittivity of the

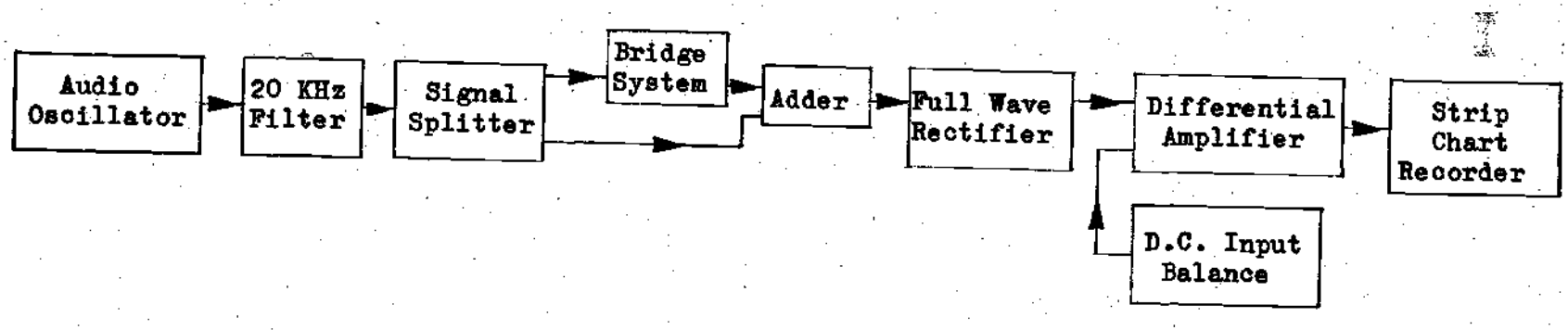


Figure 3. Block Diagram of the Electronic Measurement System.

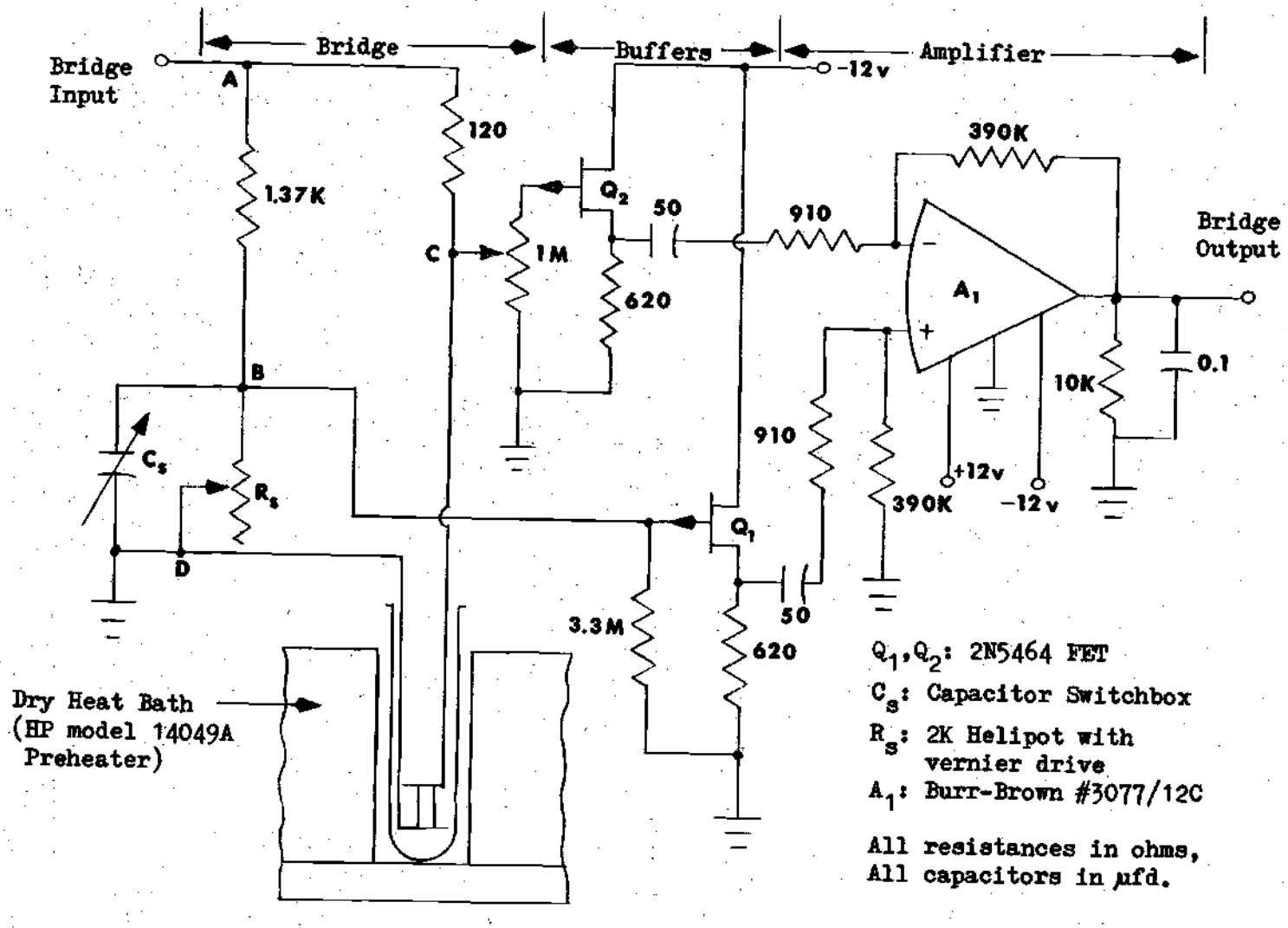
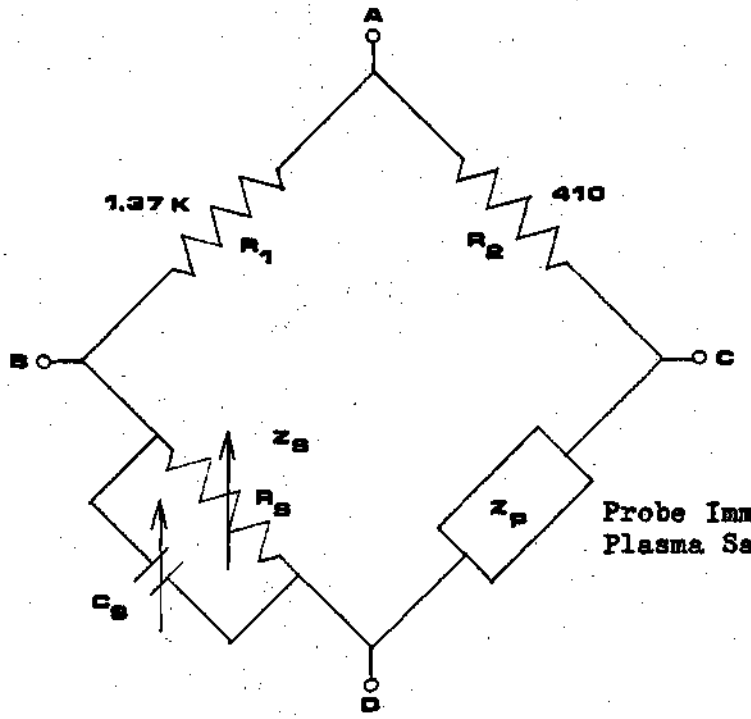
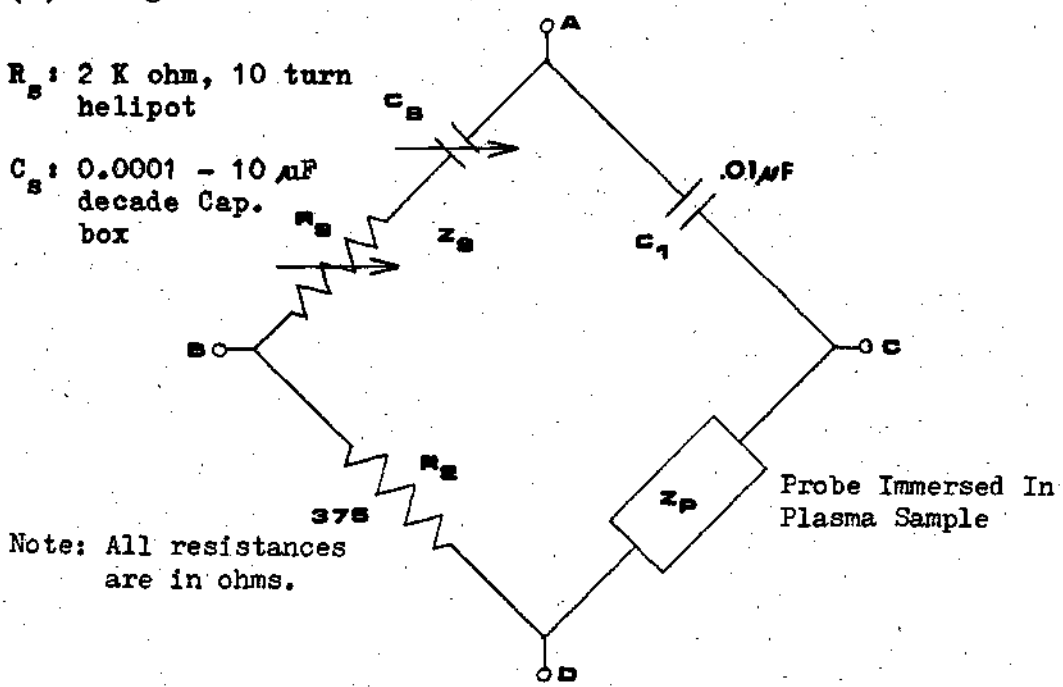


Figure 4. "Bridge System".



(a) Bridge I For Resistance Measurements.



(b) Bridge II For Capacitance Measurements.

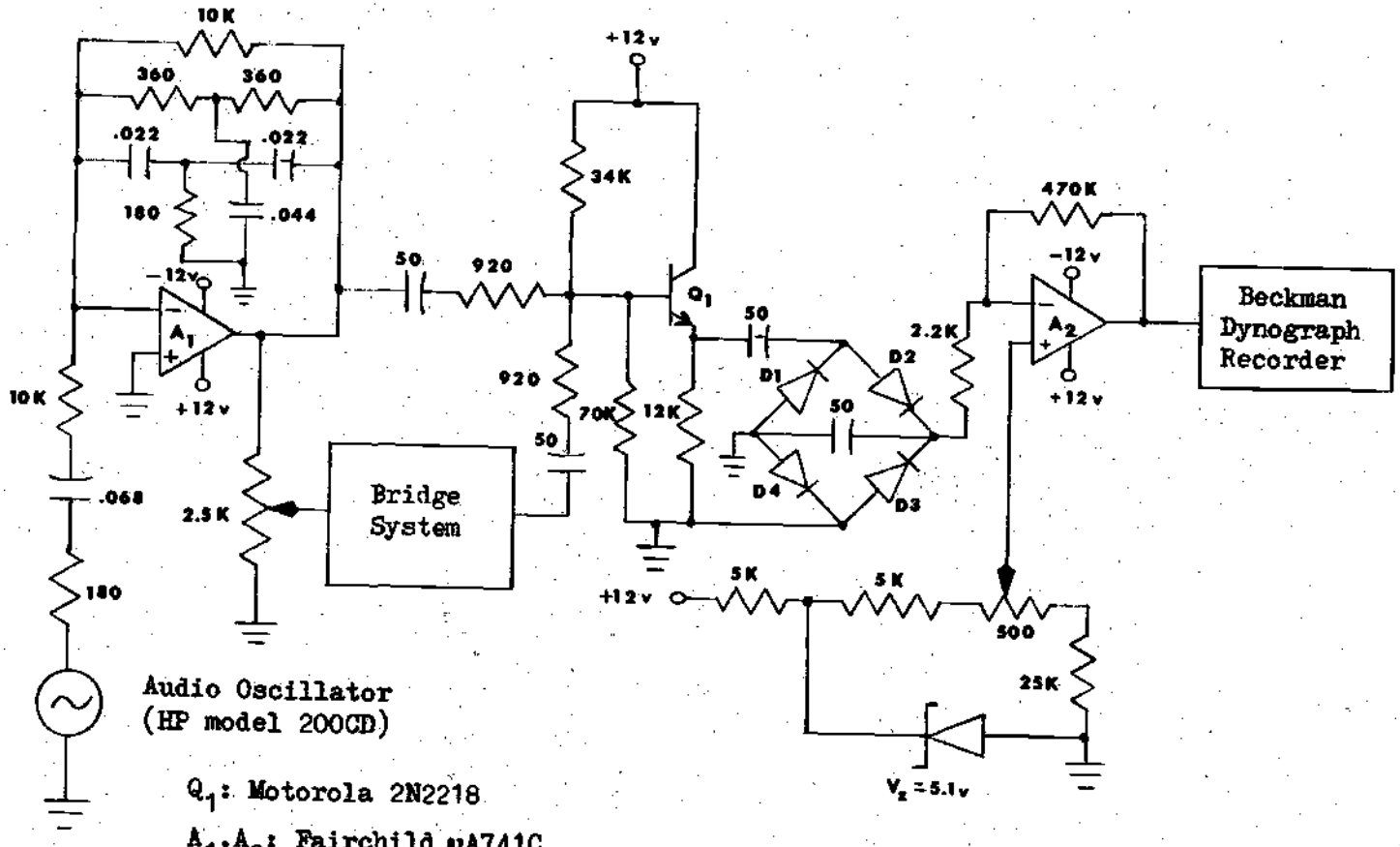
Figure 5. Bridge Components Utilized For Measurements.

plasma samples. The second signal from the signal splitter in Figure 3 is fed directly to the adder. The signal from the adder is then passed through a full wave rectifier and into a differential amplifier for amplification. A dc input signal is also fed into the differential amplifier in order to obtain a null at the beginning of a test. The signal from the differential amplifier is then recorded on a strip chart recorder. Figure 6 shows the actual electronic measurement system used in this thesis.

The input voltage level to the sample was always kept below 100 mV peak-to-peak, at 20 KHz. Since the resistance of the sample was always greater than 100 ohms, the maximum input power is

$$P = \frac{V^2}{R_p} = \frac{\left[100/2\sqrt{2} \times 10^{-3}\right]^2}{100} = 12.5 \times 10^{-6} \text{w}$$

Research reported by Lamb showed that electrolytic action in blood plasma did not take place until 20 mW of input power was applied to the sample.[18] (Since platinum-point probes were inserted in the blood veins of dogs for Lamb's work, it was not possible to determine the volume of blood subjected to electrolytic action. This precludes the possibility of making a comparison of power per unit volume between that reported by Lamb and that conducted in this study. A power density comparison would be more meaningful than the sample power comparison just cited). However, since the power delivered to the plasma samples was so small it was assumed that no significant electrolytic decomposition occurs during the measurements.



Q_1: Motorola 2N2218

A_1, A_2: Fairchild μA741C

D1, D2, D3, D4: Sylvania 1N2483

All resistances in ohms
All capacitances in μfd.

Figure 6. Electronic Measurement System.

Treatment of Samples

Whole blood samples from the medical laboratory of Piedmont Hospital in Atlanta were used in this research effort. Samples were obtained from patients at Piedmont Hospital using the venipuncture method with a Vacutainer system. This system involved the collection of whole blood samples in vacuum sealed test tubes. Each vacuum sealed test tube contained 9 mg of EDTA[19] anticoagulant to prevent coagulation. Approximately 7 cc of human blood was drawn into each test tube. The test tube was then tilted to enhance mixing of the EDTA and the human whole blood. (This lack of exact uniformity in the collection procedure of samples may account for a dispersion of the absolute values of resistivity and permittivity data obtained for these samples.) Human blood plasma was obtained from these samples by centrifuging the whole blood and EDTA at 2400 rpm for five minutes.

In order to initiate the clotting process, a calcium chloride solution was added to the EDTA treated samples. The clot time of EDTA treated samples when recalcified depends on two major factors: the quantity of $CaCl_2$ added, and the age (or length of time since acquisition) of the sample.[20] Because the exact time elapsed since acquisition was generally not known, it was necessary to make some preliminary tests to insure that the samples were not too old for accurate resistivity and permittivity measurements. The Hewlett-Packard model 1501A Coagulation Analyzer shown in Figure 7 was used to perform these preliminary tests. The use of the Coagulation Analyzer is documented in the literature.[21]

The Coagulation Analyzer is a three-channel photoelectric instrument that

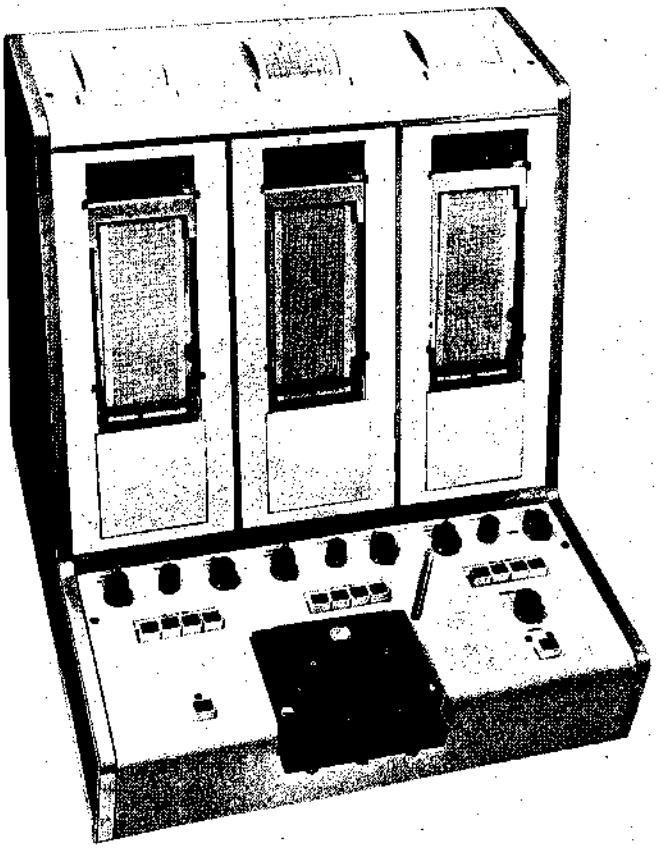


Figure 7. The Coagulation Analyzer

records blood clotting reactions continuously. The recordings provide quantitative data on the time of onset, speed of formation, and amount of fibrin formed during coagulation. The graphs also depict the start, velocity, and extent of any fibrinolysis. Neither the detecting nor recording methods affect the coagulation reactions.

Each photoelectric channel utilizes a photocell as part of a bridge circuit which is balanced to zero after introduction of a specimen so that only the change in light transmission accompanying coagulation is registered. When a sample is "old" (typically 36 hours or longer), there is very little change in the transparency during coagulation. Therefore, the Coagulation Analyzer was used to eliminate "old" samples before taking resistivity and permittivity measurements on the samples.

Environmental Effects on the Samples

Figure 8 shows the effect of temperature changes on the resistance of a plasma sample. Plasma samples were placed in a Hewlett-Packard Model 14049A Preheater set at body temperature (37°C). The accuracy of the Preheater was quoted in the specifications as being $\pm$ 0.1°C. The probe which was at 37°C was then placed in the test tube of plasma. The (Beckman Dynograph) recorder was started. The temperature of the plasma sample was allowed to increase from room temperature (24°C) to body temperature (37°C). Figure 8 shows that at t=0 the plasma sample was at 24°C and the resistance of the sample was 278 ohms. At t=4 minutes the temperature of the sample was 37°C and the resistance of the sample was 221 ohms. Geddes and Baker noted a similar decrease in resistance

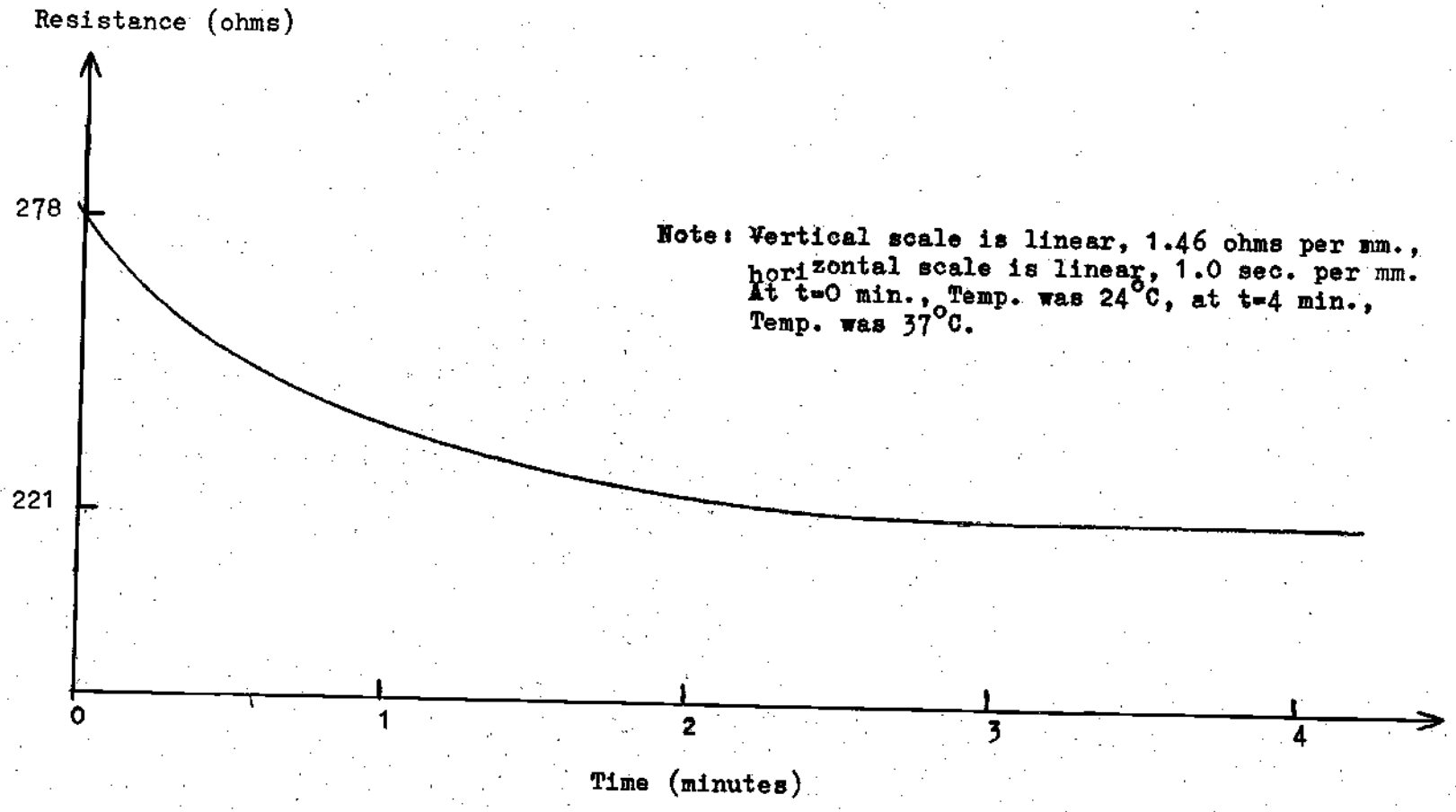


Figure 8. Temperature Effects On The Resistance Of Human Blood Plasma.

of human blood plasma with increases of temperature.[22] After approximately five minutes the thermal effect on resistance was no longer present. Hence, in all subsequent tests the samples were kept in the Preheater for at least ten minutes to allow for thermal equilibrium to be established.

Probe Effects on the Samples

Tests were conducted to check the effect of probe position in the test tube. It was observed that as long as the top plate of the probe remained below the surface of the plasma sample, the results of the resistivity and permittivity measurements were unaffected by the probe position. Thirty tests were conducted to verify this result.

In order to determine the effect of probe material on the sample, the following test was conducted. A 6 ml sample of plasma was divided into three equal parts of 2 ml each. The probe was inserted into one of the 2 ml samples. A plasma coagulogram was taken using the Hewlett-Packard Coagulation Analyzer on one of the samples without the probe (see Figure 9). A second coagulogram was taken about 30 minutes after inserting the probe, on the sample exposed to the probe (see Figure 10). Finally, a coagulogram was taken on the remaining sample not exposed to the probe (see Figure 11). The shapes of these three curves are essentially the same except for very slight differences in the amplitude and time axes. These differences are probably due to the inexactness of the amount of $CaCl_2$ added to each sample for clotting purposes. Therefore, it was assumed that the probe material has no significant effect on the coagulation of the plasma sample.

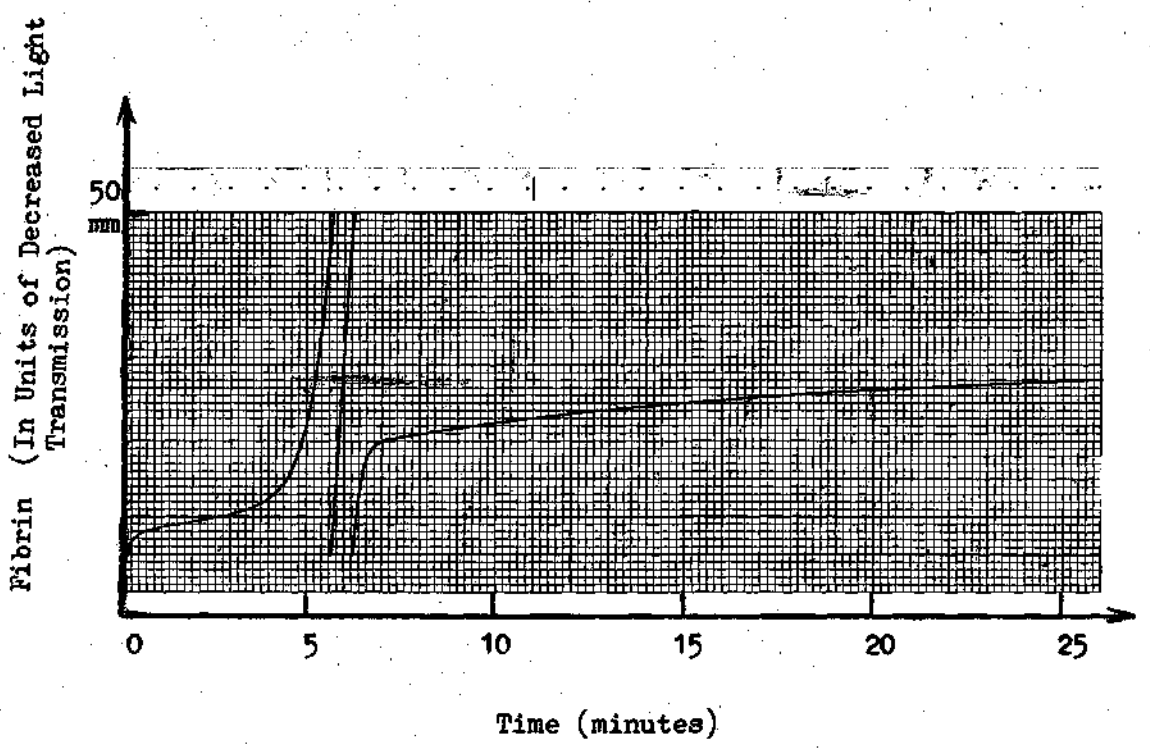


Figure 9. Coagulogram Without Sample Exposure To the Probe.

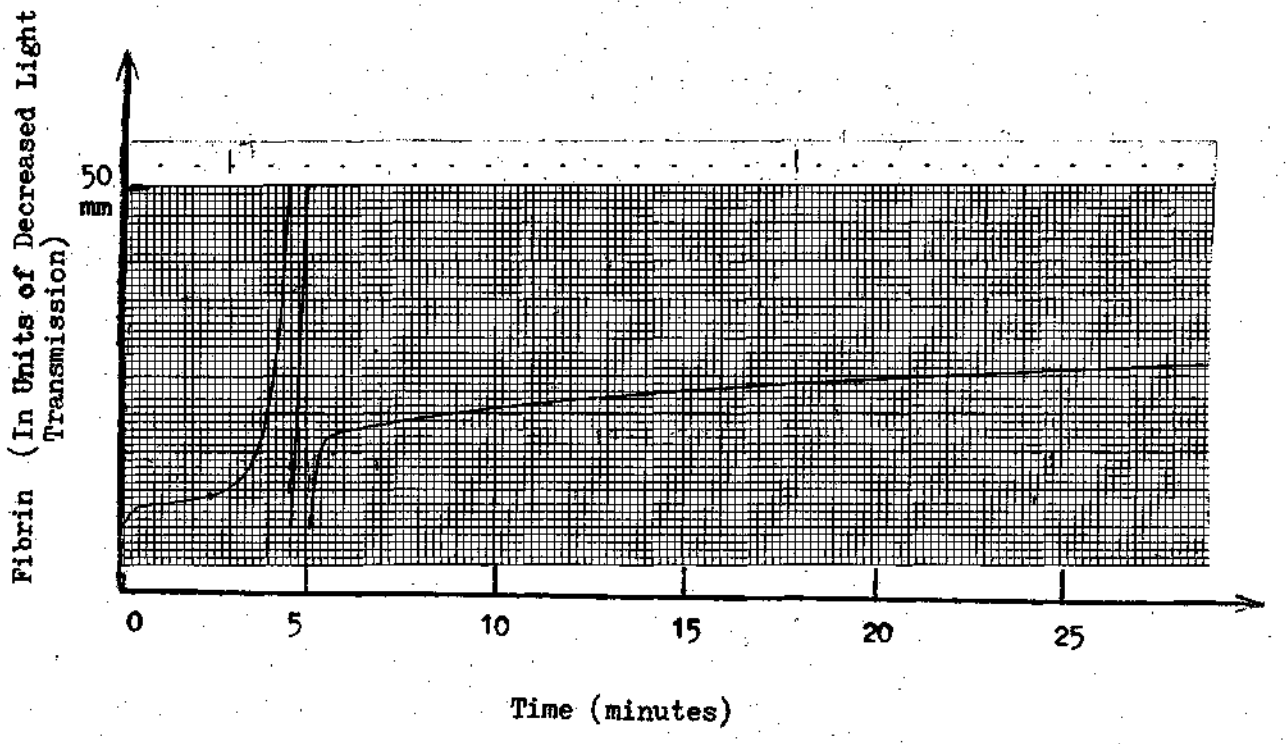


Figure 10. Coagulogram With Sample Exposure To the Probe.

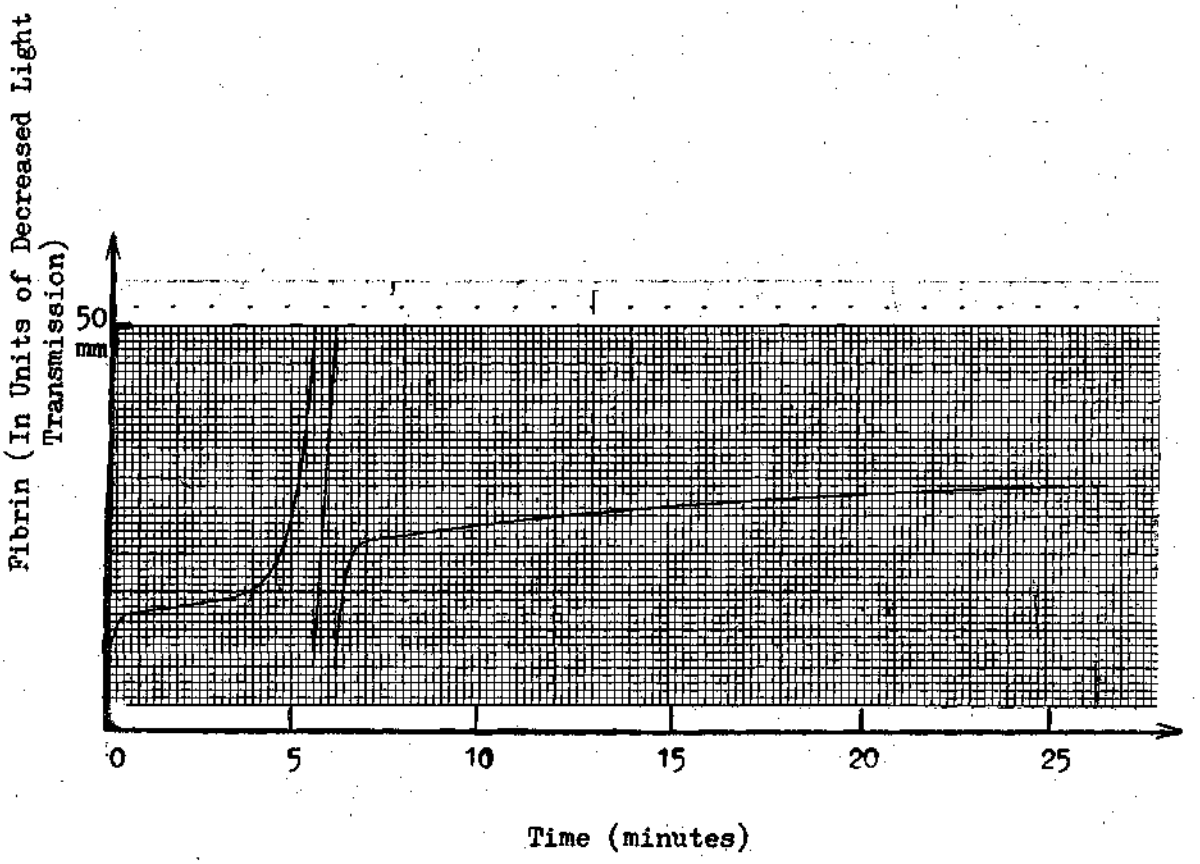


Figure 11. Coagulogram On the Remaining Sample Not Exposed To the Probe.

In order to verify that the probe did not effect the electrical parameters of the plasma samples, the following test was performed. An 8 ml sample of plasma was divided into two equal parts of 4 ml each. The two samples were then put into the Preheater and allowed to sit for ten minutes to reach thermal equilibrium. The probe (also at 37^{o}C) was then inserted into one sample and resistance and capacitance measurements were made for 30 minutes. No resistance and capacitance changes were observed during this 30 minutes. The probe was then inserted into sample number two, and resistance and capacitance measurements made. These measurements were the same measurements recorded from sample one. Therefore, it was assumed that the probe did not affect the electrical parameters of the plasma sample.

Calibration

The total accuracy of any measurement system is dependent upon the bias and precision of the measurements made with that system. The bias introduced in data taken with a measurement system can be removed by calibrating the system. Thus, a calibration check was performed on the electronic measurement system of Figure 6, for both resistivity and permittivity measurements. Of the two calibrations, the permittivity (calibration) was more critical since the effect of the plasma sample is to alter the distribution of electric flux lines. The resistivity depends only on the probe geometry, resistance of the plasma sample, and probe resistance.

As mentioned previously, the resistance of the probe in air was measured to be greater than ten meg-ohms with no plasma present. A typical value of

plasma sample and probe resistance was measured to be 250 ohms. Therefore, the presence of the plasma sample dominates the resistance measurements. Bridge I of Figure 5a was calibrated with nine known resistances (measured with a General Radio Model 1650A Impedance Bridge to an accuracy of $\pm$ 0.1 per cent). These known resistances were placed in bridge arm C-D and then measured with the electronic measurement system. The results of these tests are shown in Table 1. As seen from the plot of the data of Table 1 (Figure 12), Bridge I is calibrated for making resistance measurements.

The most critical calibration check was for the permittivity measurements made with Bridge II. Using the probe of Figure 2 in conjunction with Bridge II of Figure 5b, a calibration procedure was developed for the permittivity measurements. The range of typical values of dielectric constant of plasma samples measured was between 65.1 and 79.9. Thus for calibration purposes, it seemed appropriate to select various media having commonly accepted dielectric constants above, below, and in the same range of 65.1 to 79.9. Two media were chosen with published dielectric constants less than 65.1. These were air, 1.0, and methyl alcohol, 31.0.

A medium having a dielectric constant above 79.9, namely distilled water with a dielectric constant of 80.1 was also selected for the calibration procedure.

Finally, a .02 molar NaCl solution with a dielectric constant of 71.1 was chosen for calibration purposes, because its value of dielectric constant was in the range of the dielectric constants measured for the plasma samples, 65.1 to 79.9. All tests were performed at 37^{o}C, and with a bridge input signal of 100 mV

Table 1. Known and Measured Values of Resistance

Using Bridge I.

Test	Known Value* (ohms)	Measured Value (ohms)
1	149	150
2	201	201
3	247	246
4	298	298
5	352	351
6	401	401
7	449	450
8	497	497
9	551	550

*Measured with General Radio Model 1650A Impedance Bridge to an accuracy of ± 0.1 per cent.

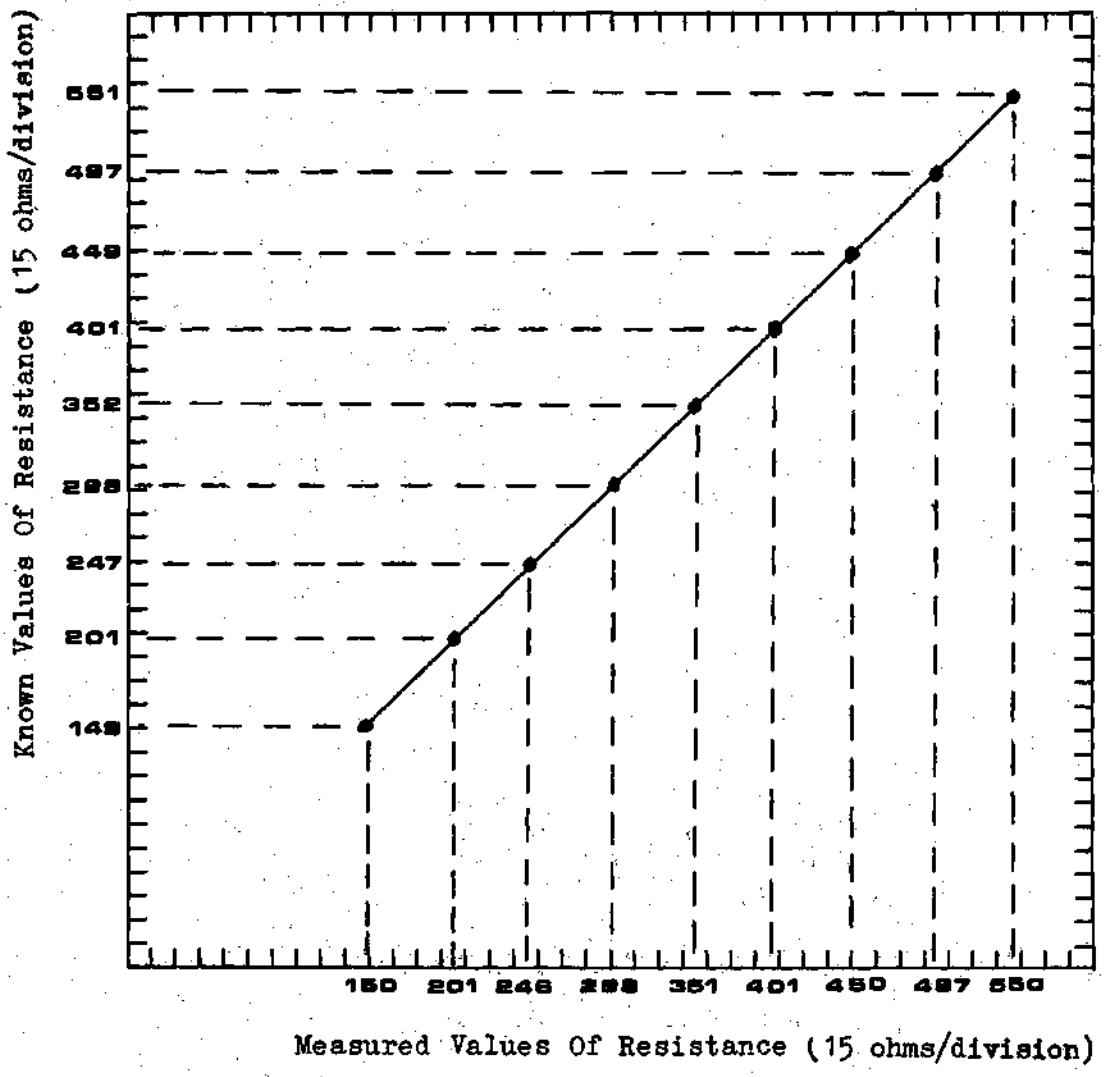


Figure 12. Known Versus Measured Values Of Resistance Using Bridge I.

peak-to-peak at 20 KHz.

The published values as well as the experimentally determined values of the dielectric constant of these four media is shown in Table 2. Figure 13 shows a graph of the measured dielectric constants versus the published values of dielectric constant for these media. Figure 13 shows that the measured dielectric constants are 18.1 larger than the published values of all four media. Therefore, it was assumed that all measurements with the experimental system that yielded dielectric constants between 19.1 and 98.2 contained a bias of + 19.1. In terms of permittivity this means that for all values of apparent permittivity measured between 1.69×10^{-12} Fd/cm (1.69×10^{-10} Fd/m, or a relative dielectric constant of 19.1) and 8.69×10^{-12} Fd/cm (8.69×10^{-10} Fd/m, or a relative dielectric constant of 98.2) there was a linear offset of $+ 1.60 \times 10^{-12}$ Fd/cm (1.60×10^{-10} Fd/m, or a relative dielectric constant offset + 18.1) which must be subtracted from the measured value of permittivity. Because this is a constant linear offset, the 18.1 offset will not effect measurements of the change in permittivity of clotting plasma samples.

Procedure

Before measurement of the plasma sample's resistance and capacitance could begin, it was necessary to allow the temperature of the test equipment, dry heat bath, and samples to stabilize. The temperature of the dry heat bath was set to 37^{o}C. All test equipment was turned on at least one hour prior to the initiation of measurements, and checked for proper settings. Resistor and capacitor standards were placed across the unknown bridge leg of Figure 5a and 5b to check

Table 2. Measured and Published Values of the Dielectric Constant for Four Different Media.

Medium	Published[a] Dielectric Constant	Measured Dielectric Constant
Distilled Water	80.1[b]	98.2
.02 Molar NACl	71.1[b]	89.2
Plasma Samples		65.1 - 79.9
Methyl Alcohol	31.0[c]	49.1
Air	1.0[c]	19.1

[a]Published data extended for 37°C and 20 KHz.

[b]Data by Saxton.[23]

[c]Data by Von Hippel.[24]

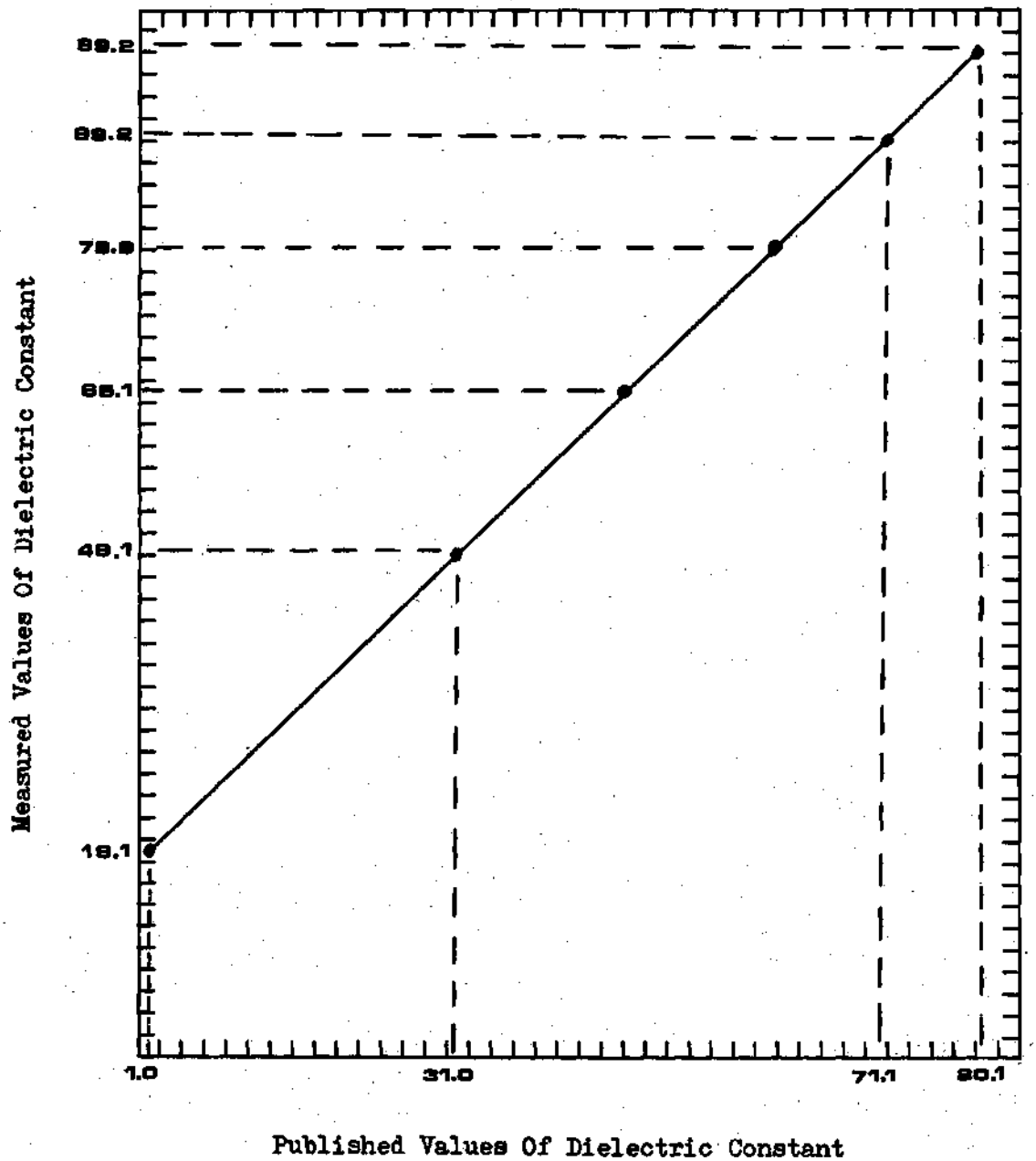


Figure 13. Experimentally Measured Values Of Dielectric Constant Versus Published Values Of Dielectric Constant For Four Different Media.

system calibration. These standards were calibrated against the General Radio Model 1650A Impedance Bridge (accuracy of ± 0.1 per cent).

The test procedure is outlined in Figure 14. Whole blood samples from the medical laboratory of Piedmont Hospital in Atlanta were used in these tests. Once the human blood containing the EDTA anticoagulant was obtained, it was centrifuged at approximately 2400 rpm for five minutes (± 6 seconds) to separate the plasma sample from the red cells. The plasma sample was then decanted off the top of the red blood cells, and divided as shown in Figure 14.

Once the divided samples had warmed to 37°C (ten minutes after immersion in the dry heat bath), then the Coagulation Analyzer test was run to test the sample for "oldness." If the sample could be made to clot as indicted by a positive deflection of at least 100 mm, on the Coagulation Analyzer recorder within five minutes, then the sample passed the "oldness" test and the resistance test was initiated. The probe was inserted in the resistive sample and Bridge I was utilized. The test tube containing the plasma sample and the probe remained in the preheater preceding and during the entire time measurements were made. Since the preheater is a solid aluminum block, it was impossible to observe any physical alteration of the sample during the testing period. Thus, physical phenomena such as color changes, air bubble pockets, etc. could not be observed during the measurements.

For the resistance test, capacitance test, and the Coagulation Analyzer test, one part of 0.02 molar $CaCl_2$ to three parts of plasma was used.[25] The $CaCl_2$ solution was warmed in the dry heat bath at 37°C before it was used to initiate coagulation. Thus, $CaCl_2$ was added to the resistive sample and the initial

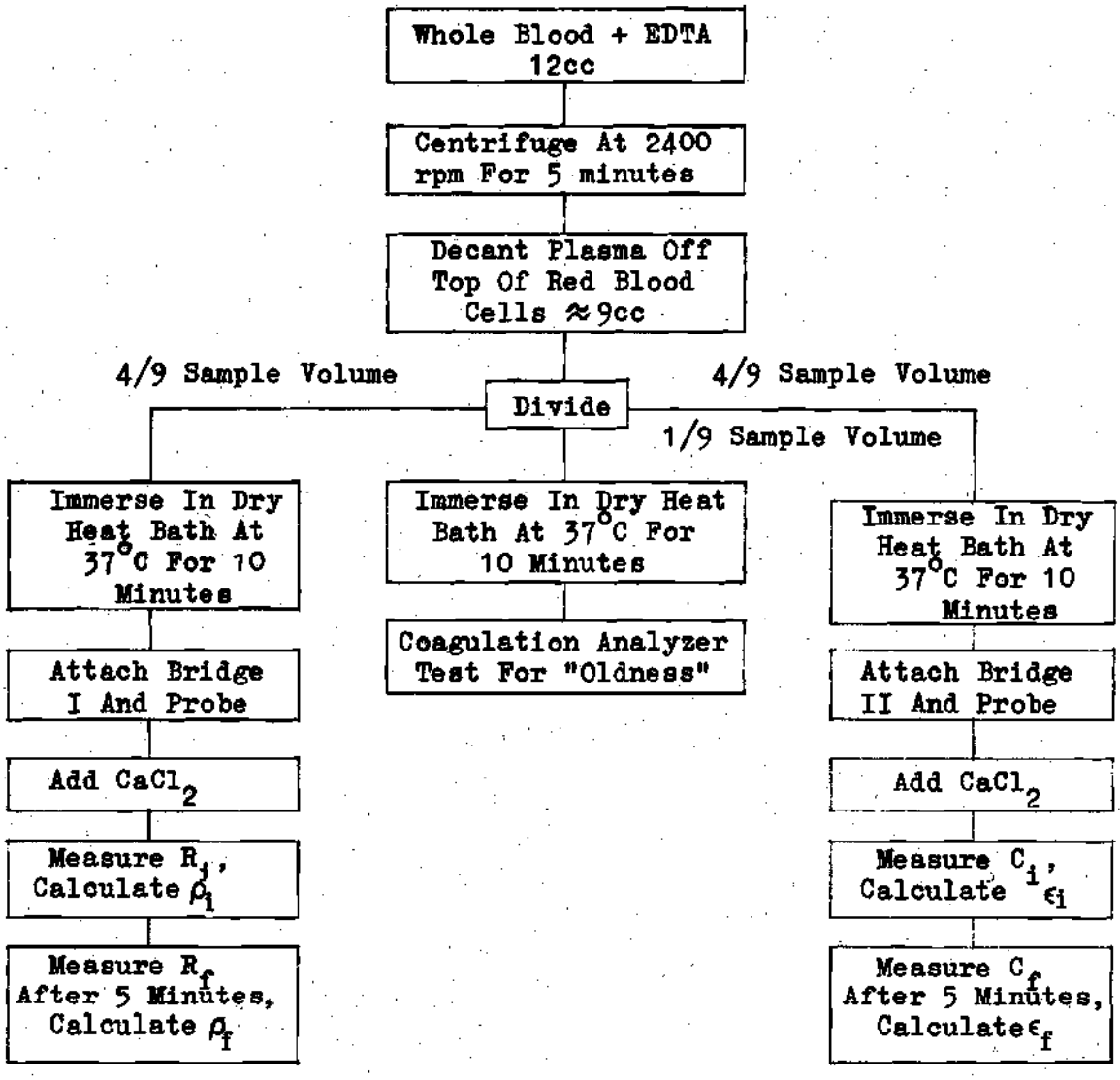


Figure 14. Test Procedure.

resistance measurement was made after the bridge system was balanced (balancing required approximately 5 to 15 seconds after the $CaCl_2$ was added to the plasma sample). The instant when the bridge system was balanced was established as the time mark of t equal zero minutes. Final values of resistance were measured and recorded at t equal five minutes after the bridge system was initially balanced.

Next, the probe was inserted into the capacitive sample and Bridge II utilized. Then $CaCl_2$ was added, and the initial capacitance measurement was made after the bridge system was balanced, establishing the time mark of t equal zero minutes. At t equal five minutes after the bridge was initially balanced, the final capacitance measurement was made. This procedure was duplicated for each of the 300 samples measured.

CHAPTER IV

EXPERIMENTAL RESULTS

Data

The reduction of the raw data into useable values of ρ and ϵ requires the computation of two conversion constants. Appendix A contains the derivation of these two constants. Appendix B contains an accuracy analysis of the bridge systems used for the resistivity and permittivity measurements. The value of ρ was derived from Bridge I and ϵ from Bridge II. In both bridges, R_s represents a 2 K ohm, 10 turn helipot, readable to three significant figures (with linearity of $\pm$ 0.2 per cent). Therefore, the resistance required for balance of the bridges, R_s, is

$$R_s = 2000\, X_s \tag{5}$$

where X_s is the scale reading on the vernier whose full scale value is 1.000.

Bridge I is the classical bridge used for accurate resistance measurements, and therefore is especially suited for measuring the plasma sample and probe combination resistance changes due to coagulation. The bridge balance equation (Equation (7a) derived in Appendix A) is:

$$Z_p = \frac{Z_2 Z_s}{Z_1} \tag{6}$$

where Z_p represents the parallel combination of R_p and C_p, i.e., the equivalent resistance and capacitance of the plasma sample and probe which comprise the unknown leg of the bridge. This equivalent model was previously presented in Figure 1, page 8. From Appendix A for Bridge I it is seen that,

$$R_p = 600\, X_s \tag{7}$$

Thus, using Equation (3), page 10, to convert R_p to an equivalent value of ρ,

$$\rho = \frac{R_p A}{L} = 600 \frac{A}{L} X_s = 152 X_s \text{ ohm-cm.} \tag{8}$$

Bridge II used for the measurement of blood plasma and probe capacitance changes due to coagulation is the well known Schering Bridge. The bridge balance Equation (25a) derived in Appendix A is:

$$Z_p = \frac{Z_1 Z_2}{Z_s} \tag{9}$$

where Z_p again represents the parallel combination of R_p and C_p. From Appendix A for Bridge II it is seen that,

$$C_p = 3.01 \times 10^{-12} X_s \text{ farads} \tag{10}$$

Thus, using Equation (4), page 10, to convert C_p to an equivalent value of ϵ,

$$\epsilon = \frac{C_p L}{A} = 11.8 \times 10^{-12} X_s \text{ Fd./cm.} \tag{11}$$

The reduced data, ρ and ϵ, is summarized in Appendix C.

Analysis of Results

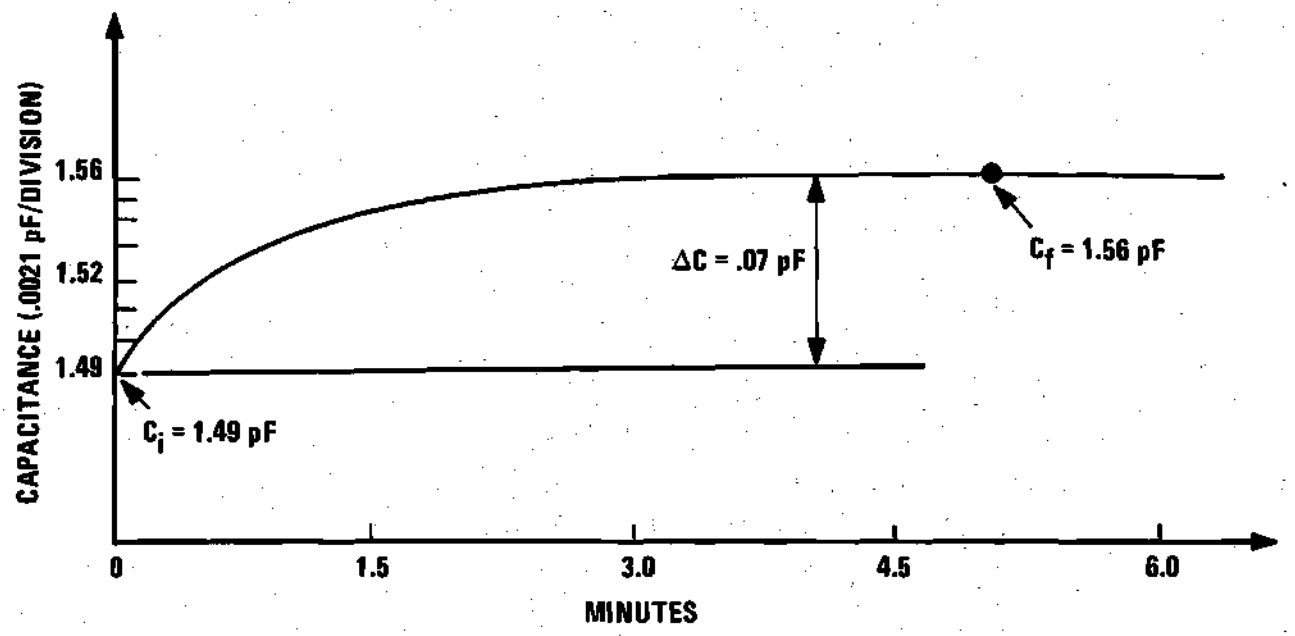
The electrical parameter changes during coagulation of a typical plasma sample (test number 53) are shown in Figure 15. For all 300 plasma samples tested, the capacitance increased monotonically as in Figure 15a. Thus, for all 300 tests the initial value of capacitance, C_i, was the smallest value for that sample and the final value of capacitance, C_f, was the largest value for that sample. Also, for all 300 samples tested, the resistance was either monotonically increasing or decreasing, where Figure 15b shows a sample for increasing resistance. Since the resistance followed a monotonic curve the initial value, R_i, and the final value, R_f, of plasma resistance were again the maximum and minimum values.

For all 300 samples tested the initial values of resistance, R_i, and capacitance, C_i, were the measurements taken approximately 5 to 15 seconds after the addition of the $CaCl_2$ to the human blood plasma (the time needed to manually balance the bridge). Final values of resistance, R_f, and capacitance, C_f, were taken five minutes after the bridge system was initially balanced.

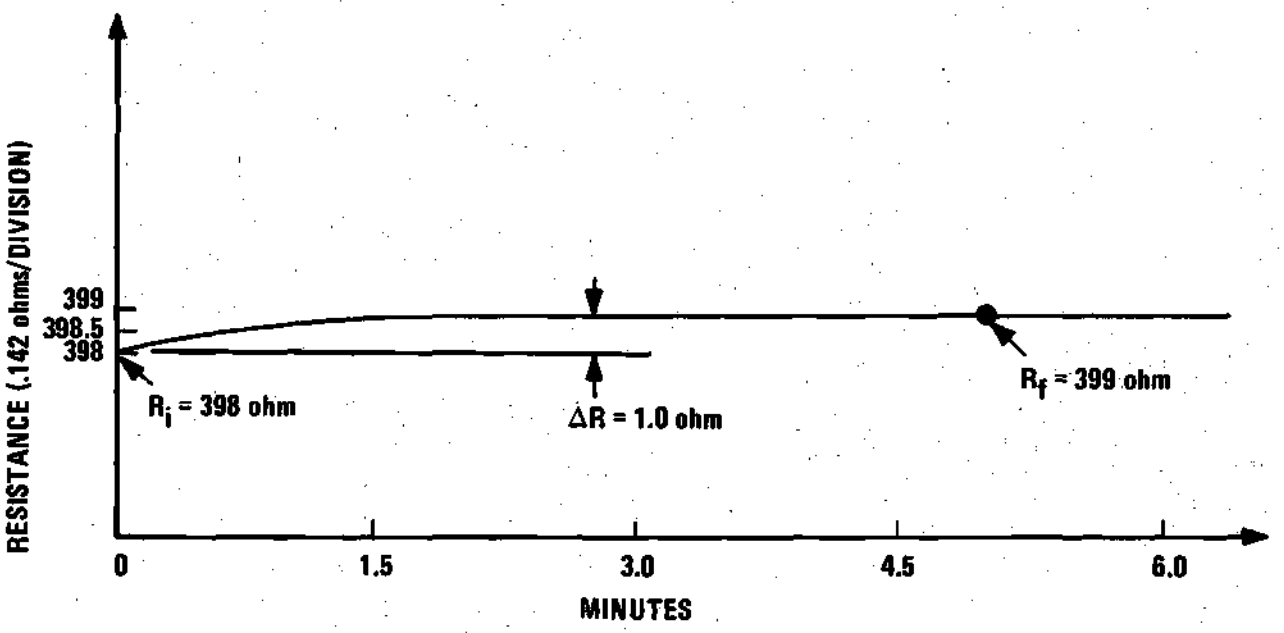
In order to determine the effects of coagulation upon the resistivity of plasma samples, a change in resistivity was defined as:

$$\Delta\rho = \rho_f - \rho_i \tag{12}$$

where ρ_f is the value of resistivity five minutes after the initiation of coagulation,



a) Typical Capacitance Change of Plasma Sample During Coagulation.



b) Typical Resistance Change of Plasma Sample During Coagulation.

Figure 15. Typical Resistance and Capacitance Variations of Plasma Samples During Coagulation.

and ρ_1 is the value of resistivity before coagulation (Figure 14, showed the procedure in making the measurements). Figure 16 shows a histogram of the changes in resistivity of plasma samples before and after coagulation for the 300 samples tested. Values of $\Delta\rho$ were placed on the histogram in the following manner. Intervals of .25 ohm-cm were selected for quantizing the changes in resistivity. If the change in resistivity was between .50 ohm-cm and .74 ohm-cm inclusive, then $\Delta\rho$ was placed in the .50 ohm-cm column of the histogram. All points of resistivity change were treated in a similar manner.

The average change in resistivity during coagulation was defined as:

$$\overline{\Delta\rho} = \frac{\sum_{x=1}^{N} (\Delta\rho)_x}{N} = \frac{\sum_{x=1}^{N} (\rho_f - \rho_i)_x}{N} \qquad (13)$$

where N is the total number of tests (300 in this case). In order to determine the statistical significance of the $\Delta\rho$ data, the standard deviation of the change in resistivity was found from:

$$\sigma_{\Delta\rho} = \frac{\sum_{x=1}^{N} (\Delta\rho - \overline{\Delta\rho})_x^2}{N-1} \qquad (14)$$

where $\sigma_{\Delta\rho}$ is the standard deviation of the resistivity change about its mean $\overline{\Delta\rho}$. The variance of $\Delta\rho$ can be found by squaring the standard deviation. From the data of Appendix C, the mean value of $\overline{\Delta\rho}$ during coagulation was calculated using Equation (13) to be .04 ohm-cm. The standard deviation was calculated using

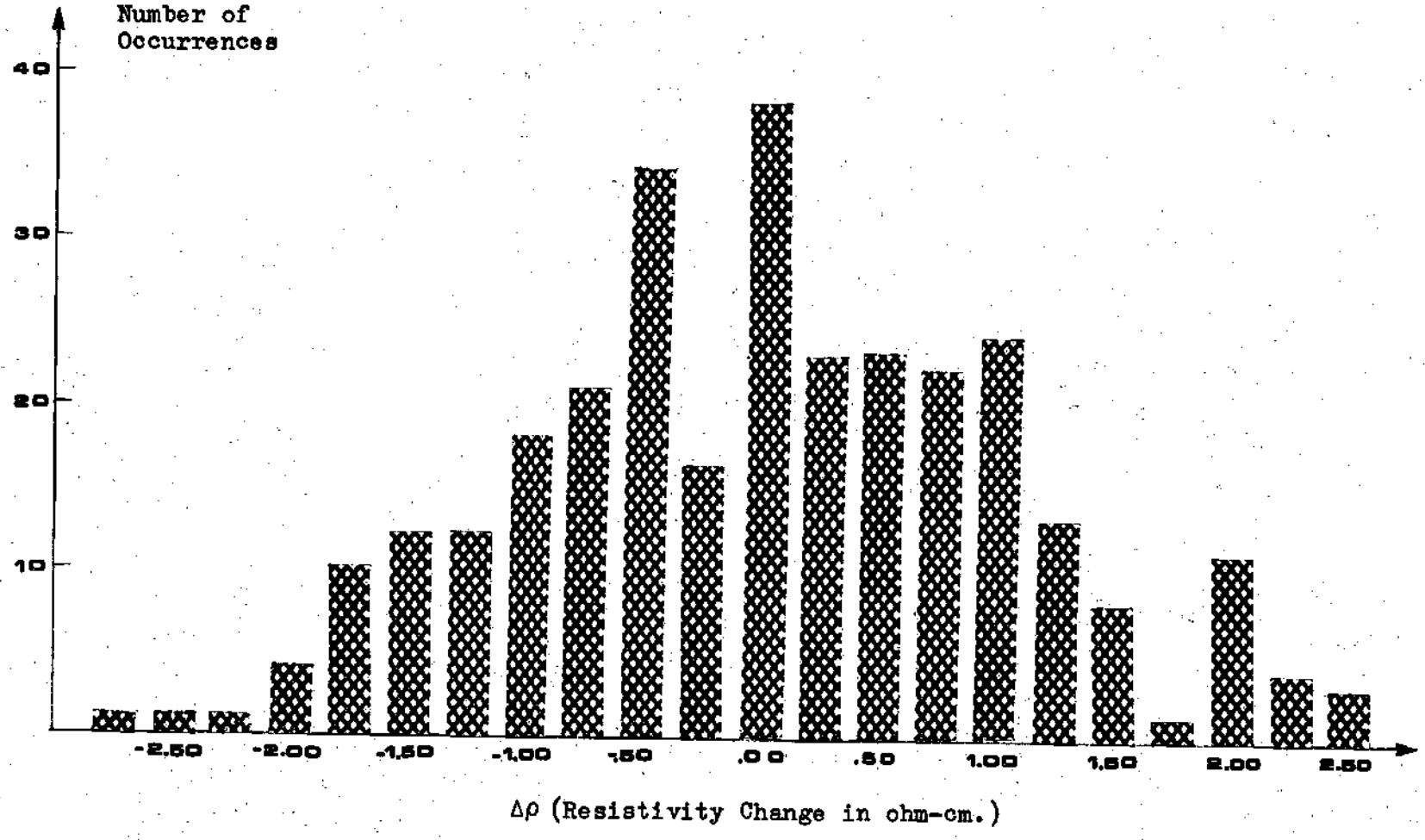


Figure 16. Histogram Showing the Frequency of Occurrence of Changes in Resistivity.

Equation (14) to be 1.03 ohm-cm. The variance of $\Delta\rho$ was 1.06 $(\text{ohm-cm})^2$.

Figure 17 shows a histogram of the changes in permittivity of human blood plasma during coagulation. Points were located on this $\Delta\epsilon$ histogram in a manner similar to that used for Figure 15. In order to determine the effects of coagulation upon the permittivity of plasma samples, a similar analysis was carried out for the permittivity changes, where:

$$\Delta\epsilon = \epsilon_f - \epsilon_i \tag{15}$$

$$\overline{\Delta\epsilon} = \frac{\sum_{x=1}^{N} (\Delta\epsilon)_x}{N} = \frac{\sum_{x=1}^{N} (\epsilon_f - \epsilon_i)_x}{N} \tag{16}$$

$$\sigma_{\Delta\epsilon} = \frac{\sum_{x=1}^{N} (\Delta\epsilon - \overline{\Delta\epsilon})_x^2}{N - 1} \tag{17}$$

and

$$\sigma_{\Delta\epsilon}^2 = \text{variance of } \Delta\epsilon \tag{18}$$

Using the data of Appendix C, and Equations (15), (16), (17), and (18) then:

$$\overline{\Delta\epsilon} = .20 \text{ pF/cm} \tag{19}$$

$$\sigma_{\Delta\epsilon} = .10 \text{ pF/cm} \tag{20}$$

and

$$\sigma_{\Delta\epsilon}^2 = .01(\text{pF/cm})^2 \tag{21}$$

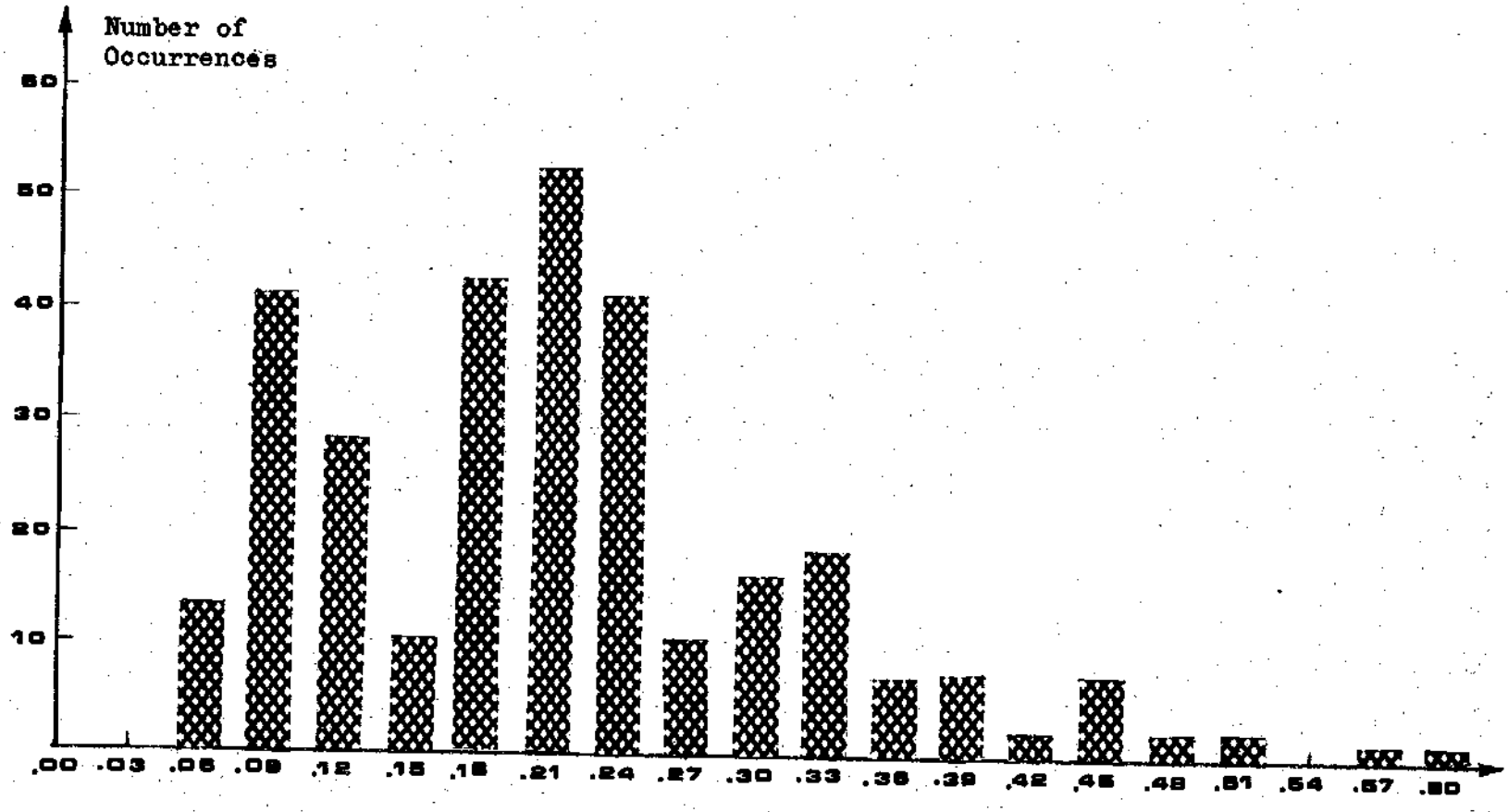


Figure 17. Histogram Showing the Frequency of Occurrence of Changes in Permittivity.

In order to determine if there was any correlation between the resistivity and permittivity changes for the 300 samples tested, the correlation coefficient of the resistivity and permittivity changes was calculated. The 300 values of $\Delta\rho$ and the 300 values of $\Delta\epsilon$ were treated as two separate stationary sample series. Thus, the correlation coefficient between the two series is defined as (similar to equations developed in Lathi[26]):

$$r_{\Delta\rho\,\Delta\epsilon} = \frac{K_{\Delta\rho\,\Delta\epsilon}}{\sigma_{\Delta\rho}\sigma_{\Delta\epsilon}} \tag{22}$$

where $\sigma_{\Delta\rho}$ and $\sigma_{\Delta\epsilon}$ are the standard deviations of $\Delta\rho$ and $\Delta\epsilon$, respectively; and $K_{\Delta\rho\,\Delta\epsilon}$ is the covariance of $\Delta\rho$ and $\Delta\epsilon$. The covariance, $K_{\Delta\rho\Delta\epsilon}$, is given by the mean of $(\Delta\rho - \overline{\Delta\rho})(\Delta\epsilon - \overline{\Delta\epsilon})$.

The correlation coefficient between the variables $\Delta\rho$ and $\Delta\epsilon$, always lies in the range of $-1 \leq r_{\Delta\rho\Delta\epsilon} \leq 1$. The maximum amount of correlation ($r_{\Delta\rho\Delta\epsilon} = \pm 1$) occurs if the variables are linearly related. The minimum amount of correlation ($r_{\Delta\rho\Delta\epsilon}$) exists when the two variables are uncorrelated.

A computer program was written in Fortran IV language for the Univac 1108 computer (using subroutine AUXCOR[27]) for computing the correlation coefficient of resistivity and permittivity changes, and is given in Appendix D.

Using the program of Appendix D it was found that $r_{\Delta\rho\Delta\epsilon}$ equals .0155. It is apparent that the correlation between the $\Delta\rho$ and $\Delta\epsilon$ data for the 300 tests performed was very close to zero as compared to $\pm$ 1. Thus, there was no apparent correlation between the $\Delta\rho$ and $\Delta\epsilon$ data taken for the 300 plasma samples tested.

Conclusions on Experimental Results

The permittivity of all 300 samples tested increased during coagulation. The accuracy of the measurement system incorporating Bridge II to measure permittivity changes was calculated in Appendix B to be $\pm$ .5 per cent. The average percentage change in permittivity for the 300 samples was +3.19 per cent, which is more than six times greater than the accuracy of the system. Thus, the changes in permittivity observed were well within the accuracy limits of Bridge II. From Equations (19) and (20) the average increase in permittivity during coagulation was +.20 pF/cm (20 pF/m or a relative dielectric constant change of 2.26) with a standard deviation about $\overline{\Delta\epsilon}$ of .10 pF/cm. Thus, since there were no decreases in permittivity, 99.7 per cent of the 300 samples tested had a permittivity increase of .06 to .50 pF/cm. Since the standard deviation of $\Delta\epsilon$, ($\sigma_{\Delta\epsilon}$ = .10 pF/cm), is of the same order of magnitude as $\overline{\Delta\epsilon}$, ($\overline{\Delta\epsilon}$ = +.20 pF/cm), the spread of the experimental data is too large to warrant firm conclusions as to the average value of permittivity change before and after coagulation.

No uniform positive or negative trend of resistivity changes was apparent from the 300 samples tested. The accuracy of the measurement system utilizing Bridge I was calculated to be $\pm$ .5 per cent. The average percentage change in resistivity was +.05 per cent. Since the error associated with the resistivity measurement is ten times the average percentage change, the average resistivity of the 300 samples remains relatively constant (less than $\pm$ .5 per cent) during the coagulation process. Measurement of changes less than $\pm$ .5 per cent are due to a lack of sensitivity in the measurement system for detecting resistive

variations due to coagulation. The experimental data does indicate that no resistive variation greater than 3.6 per cent was evident during the 300 test runs.

There was no apparent correlation between the $\Delta\rho$ and $\Delta\epsilon$ data obtained for the 300 blood plasma samples tested. The correlation coefficient of the $\Delta\rho$ and $\Delta\epsilon$ data was .0155 which is very close to zero (two variables uncorrelated) as compared to $\pm$ 1 (two variables linearly related).

CHAPTER V

CONCLUSIONS

Coagulation causes the permittivity of plasma samples to increase. The average increase in the permittivity of plasma samples due to coagulation is significant and exceeded three per cent of the initial average value measured. This change is not attributable to inaccuracies in Bridge II, since the bridge accuracy is six times better than the three per cent average permittivity change due to coagulation. Thus, this average change in permittivity of human blood plasma-$CaCl_2$ mixtures must be associated with coagulation effects. Of the 300 samples tested 99.7 per cent had an increase of permittivity from .06 to .50 pF/cm. The average increase in permittivity was +.20 pF/cm, and the standard deviation of the permittivity changes was .10 pF/cm. Thus, since the standard deviation of $\Delta\epsilon$ is of the same order of magnitude as $\overline{\Delta\epsilon}$, the spread of the experimental data is too large to warrant firm conclusions as to the average value of permittivity change.

No uniform positive or negative trend was apparent from the resistivity changes of the 300 plasma samples tested. The error of Bridge I was ten times the average percentage resistivity change of .05 per cent. Thus, the average resistivity of the 300 samples remains relatively constant (less than $\pm$ .5 per cent change) during the coagulation process. Measurement of changes less than $\pm$.5 per cent are due to a lack of sensitivity in the measurement system. The

experimental data revealed that no resistive variation greater than 3.6 per cent was evident during the 300 test runs.

Calculation of the correlation coefficient between the resistivity and permittivity changes indicated that there was no apparent correlation between resistivity and permittivity variations due to the coagulation of human blood plasma. The correlation coefficient between the resistivity and permittivity changes for the 300 samples tested was .0155.

CHAPTER VI

RECOMMENDATIONS FOR FURTHER STUDY

The results of this work suggest many areas of further study. Seven of the most promising of these are discussed below.

1. The literature search did not discover any studies on chemical changes that cause electrical parameter variations due to coagulation of human blood plasma. Original research dealing with electrical-chemical phenomena of blood might reveal some electrical parameter variation traceable to the chemical changes of blood during coagulation.

2. It was observed at the medical laboratory of the Piedmont Hospital in Atlanta that clot time data was taken using the Lee White method, without regard to the temperature variation of the sample. (The Lee White test consists of tipping test tubes of plasma on their sides every 30 seconds to determine the clot time of the sample). By further study of the effects of temperature variations on the electrical parameters of human blood plasma it may be possible to prove that clot time data taken without regard to temperature variations is highly inaccurate.

3. The blood samples used in this study had no known medical history report of the patient. Using samples from patients with known medical histories could reveal a correlation between medical history of the sample and permittivity and/or resistivity variations.

4. A study of the frequency dependence of the dielectric constant of human

blood plasma during coagulation might reveal frequency shifts in the dielectric constant curve directly related to the coagulation process. This shift in frequency could be important because of its possibilities of predicting clot times and/or disease characteristics of human blood.

5. Possible sources of error in this work were: amount of EDTA anti-coagulant added to each human blood sample, accuracy of concentration of EDTA, length of time the sample was centrifuged, speed at which the sample was centrifuged, length of time needed to balance the bridge system before making initial measurements, temperature accuracy of the dry heat bath, accuracy of combining 75 per cent human blood plasma and 25 per cent $CaCl_2$, room temperature deviations, air bubbles after inserting the probe into the sample, and actual deviations in protein content from one human blood sample to another. Any of these effects could be studied in depth to determine their effects on the electrical characteristics of the samples.

6. Another possible area of investigation, would be to perform the same tests of this work using a probe of cylindrical geometry, to note the effects of probe geometry on the results.

7. Physicians use many different procedures to measure the clotting characteristics of blood. Before information on the electrical characteristics of blood during coagulation can be of value in determining human blood plasma clot times, it must be correlated with existing measurement methods, perhaps by comparing clot times obtained with electrical procedures to times determined by presently used clinical methods, or by using standard solutions which clot at a

previously determined point. One important application of the electrical technique for the determination of clot times is for in vivo measurements. Continuous, in vivo measurements would be extremely important for monitoring the rate and extent of clot formation surrounding artificially implanted arterial and veneous tubing within the circulatory system.

APPENDIX A

BRIDGE BALANCE EQUATIONS

A generalized four arm a.c. bridge is shown in Figure A1. If E_d, the detector voltage is zero, the bridge is balanced[26]. Under this condition,

$$V_1 = V_2. \tag{1a}$$

Balance conditions for the bridge are found by equating products of impedances in opposite arms. Thus,

$$Z_p Z_b = Z_c Z_a \tag{2a}$$

Equation (2a) is known as the balance equation.

Bridge I

The first step in computing the conversion constant for the probe and sample resistance (R_p of Figure 5a), involves the use of the bridge balance Equation (2a). From Figure 5a (Bridge I), page 14, it is seen that:

$$Z_p = Z_p \tag{3a}$$

$$Z_b = Z_1 \tag{4a}$$

$$Z_c = Z_2 \tag{5a}$$

$$Z_a = Z_s \tag{6a}$$

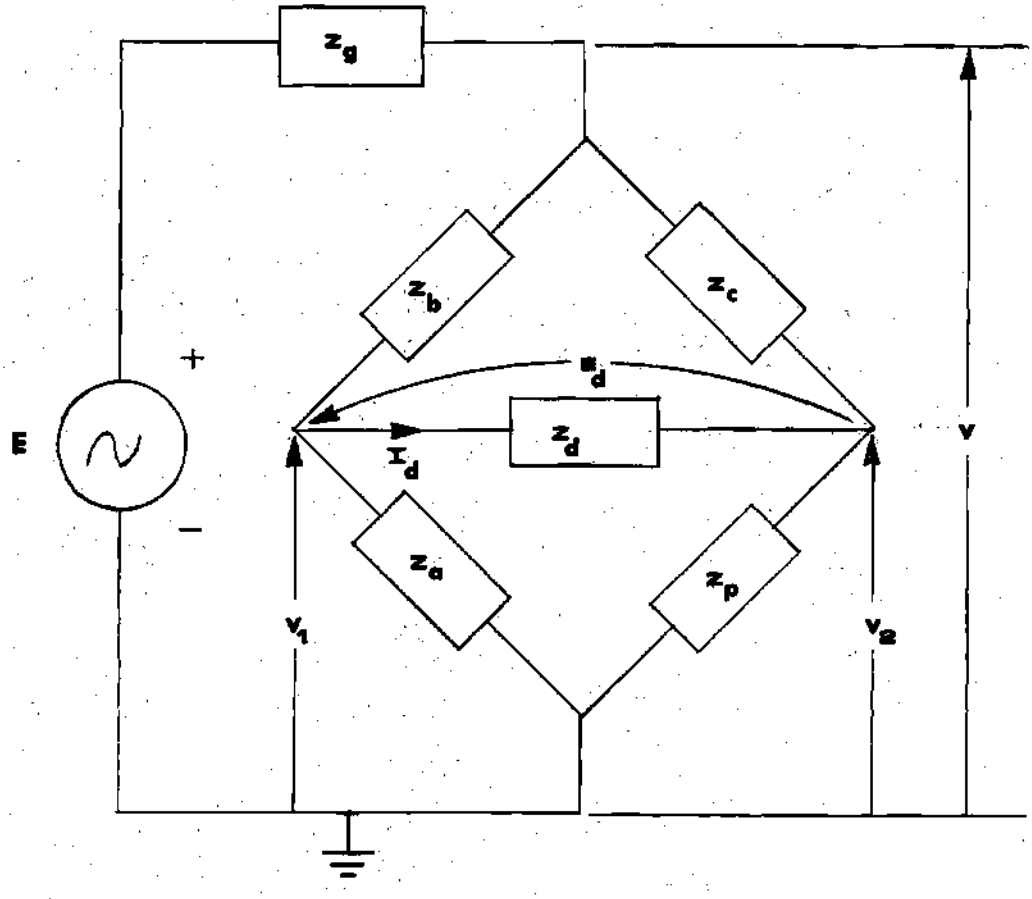


Figure A1. Generalized Four-Arm A.C. Bridge.

Thus Equation (2a) becomes,

$$Z_p = \frac{Z_2}{Z_1} Z_s \tag{7a}$$

or

$$\frac{1}{Y_p} = \frac{Z_2}{Z_1} \frac{1}{Y_s} \tag{8a}$$

Z_2/Z_1 is a constant ratio of two resistances, therefore, Equation (8a) becomes,

$$\frac{1}{G_p + jB_p} = \frac{K}{G_s + jB_s} \tag{9a}$$

where

$$K = Z_2/Z_1 \tag{10a}$$

Rearranging Equation (9a) gives,

$$G_p + jB_p = \frac{1}{K} (G_s + jB_s) \tag{11a}$$

equating real and imaginary parts of Equation (11a),

$$G_p = G_s/K \tag{12a}$$

$$B_p = B_s/K \tag{13a}$$

since

$$B_p = WC_p \tag{14a}$$

$$B_s = WC_s \tag{15a}$$

$$G_p = 1/R_p \tag{16a}$$

$$G_s = 1/R_s \tag{17a}$$

then Equations (12a) and (13a) become,

$$R_p = R_s K \tag{18a}$$

$$C_p = C_s K \tag{19a}$$

Since K is a constant, the R_p and C_p balance equations are mutually exclusive and independent of frequency for bridge balance. Using the component values in Figure 5a, page 14, (measured with the General Radio model 1650A Impedance Bridge to an accuracy of $\pm$ 0.1 per cent) to compute K and Equation (5), then:

$$R_p = 600\, X_s \text{ ohms} \tag{20a}$$

Bridge II

From Figure 5b, page 14, (Bridge II), it is seen that:

$$Z_p = Z_p \tag{21a}$$

$$Z_b = Z_s \tag{22a}$$

$$Z_c = Z_1 \tag{23a}$$

$$Z_a = Z_2 \tag{24a}$$

Thus Equation (2a) becomes,

$$Z_p = \frac{Z_1 Z_2}{Z_s} \tag{25a}$$

From Figure 5b,

$$Z_p = \frac{R_p}{1 + jwC_pR_p} \tag{26a}$$

$$Z_1 = 1/jwC_1 \tag{27a}$$

$$Z_2 = R_2 \tag{28a}$$

$$Z_s = R_s + \frac{1}{jwC_s} \tag{29a}$$

Substituting Equations (26a), (27a), (28a), and (29a) into Equation (25a) gives,

$$\frac{R_p}{1+jwC_pR_p} = \frac{\left(\frac{1}{jwC_1}\right)\left(R_2\right)}{R_s+\left(\frac{1}{jwC_s}\right)} \tag{30a}$$

Rearranging Equation (30a) gives,

$$C_1R_p + jwR_sC_sC_1R_p = R_2C_s + jwC_pR_pC_sR_2 \tag{31a}$$

Equating real and imaginary parts of Equation (31a),

$$R_p = \frac{R_2}{C_1}\ C_s \tag{32a}$$

$$C_p = \frac{C_1}{R_2}\ R_s \tag{33a}$$

Since R_2 and C_1 are constant values, then R_p and C_p are mutually exclusive and

independent of frequency for bridge balance. Bridge II was used to measure the capacitance of the blood plasma and probe, C_p, Equation (33a). C_1/R_2 is a constant ratio for Bridge II, thus Equation (33a) becomes,

$$C_p = R_s K_1 \tag{34a}$$

where

$$K_1 = C_1/R_2 \tag{35a}$$

Substituting Equation (5) into (34a) gives,

$$C_p = 2000\, K_1 X_s \tag{36a}$$

Using the component values shown in Figure 5b, (measured to $\pm$ 0.1 per cent accuracy with the General Radio model 1650A Impedance Bridge), to compute K_1, then:

$$C_p = 3.01 \times 10^{-12} X_s \text{ farads} \tag{37a}$$

APPENDIX B

ACCURACY ANALYSIS OF THE BRIDGE SYSTEMS

The analysis of the a.c. bridge systems to determine the accuracy of the measurements involves two sources of error:

1. Bias -- the systematic error that is the same for each measurement and thus can be removed by calibration.
2. Precision--the random error, or nonrepeatability. It is, in general, different for every measurement, and can only be bounded but cannot be removed completely. Precision is directly attributable to sensitivity and read-out error of the measurements.

Sensitivity

The sensitivity, S, is given mathematically as:

$$S = \left| \frac{\Delta E_d / E}{\Delta Z_p / Z_p} \right| \tag{38a}$$

where ΔE_d is defined as a change in the detector voltage from the balance condition, per unit source voltage, E, produced by a fractional change in the impedance of the unknown arm, ΔZ_p, as measured from the impedance value for exact balance, Z_p.[27]

The sensitivity, S, is a function of the six bridge system components Z_g,

Z_p, Z_d, Z_a, Z_b, and Z_c shown in Figure A1, page 52. A small change of ΔZ_p in the arm Z_p produces a small voltage unbalance in the bridge E_d, which is sensed by the detector. A complete solution for the detector voltage, ΔE_d may be obtained by use of conventional circuit analysis, where ΔE_d is found to be:[28]

$$\Delta E_d = \frac{E Z_d}{\left(Z_p+Z_g+Z_c+\frac{Z_g Z_c}{Z_b}\right)\left(Z_d+Z_a+Z_p+\frac{Z_d Z_a}{Z_b}\right)} \cdot \frac{\Delta Z_p}{1+K\Delta Z_p} \qquad (39a)$$

where

$$K = \frac{Z_b(Z_c+Z_a) + (Z_g+Z_a)(Z_b+Z_c+Z_d)}{Z_b\left(Z_g+Z_c+Z_p+\frac{Z_g Z_c}{Z_b}\right)\left(Z_d+Z_a+Z_p+\frac{Z_d Z_a}{Z_b}\right)} \qquad (40a)$$

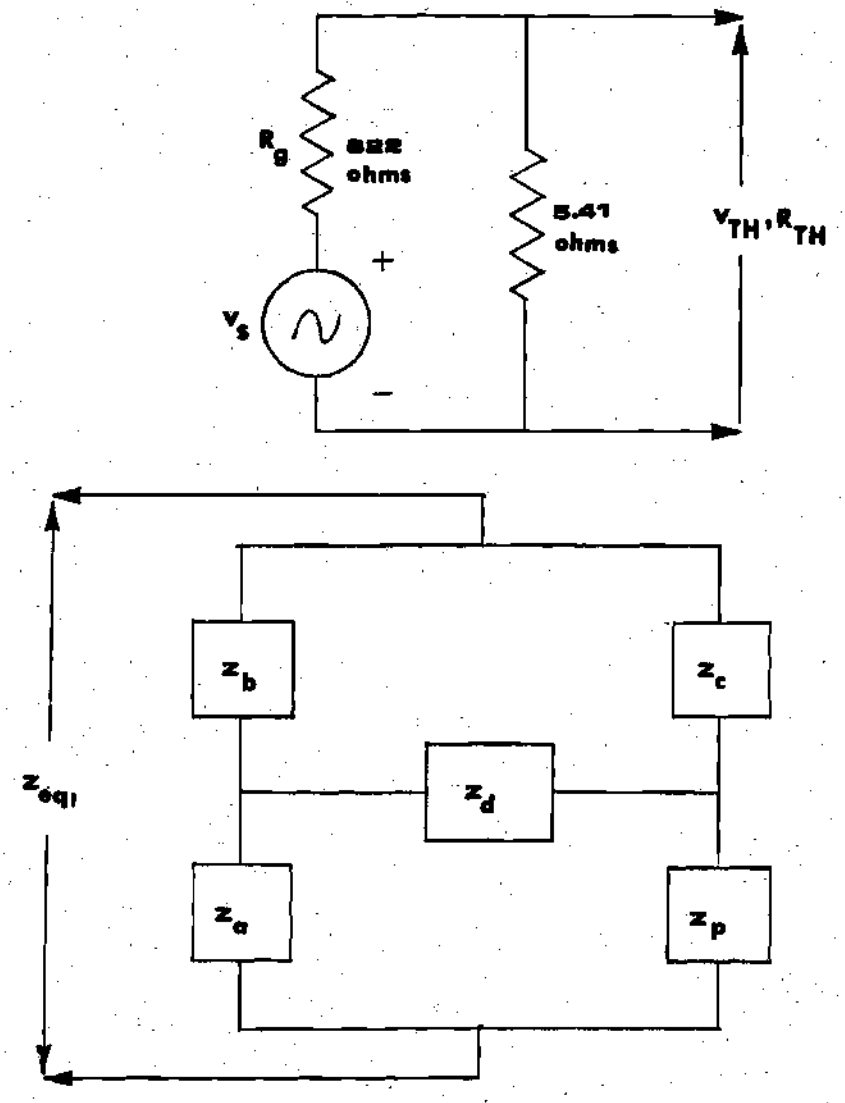
If ΔZ_p is very small compared to Z_p, then $K\Delta Z_p \ll 1$ and can be neglected with respect to one. To analyze the two bridges in question using $K\Delta Z_p \ll 1$ it is necessary to show that the ideal assumptions of $Z_g = 0$ and $Z_d = \infty$ have been approximated to a sufficient degree.

The assumption that $Z_g = 0$ is equivalent to showing that $Z_g \ll Z_{eq1}$ the impedance "seen" by the generator. Applying Thevenin's theorem in the manner shown in Figure A2,

$$V_{TH} = \frac{5.41\ V_s}{5.31+622} = .0086\ V_s \qquad (41a)$$

$$R_{TH} = \frac{(5.41)(622)}{5.41+622} = 5.41 \text{ ohms} \qquad (42a)$$

The Thevenin resistance, R_{TH}, is the equivalent impedance of the generator, Z_g,



In terms of Figure A1, page 52;

$$E = V_{TH} = \frac{5.4}{5.4 + 622} V_s = .0086\ V_s$$

$$Z_g = R_{TH} = \frac{5.4(622)}{5.4 + 622} = 5.4 \text{ ohms}$$

Figure A2. Equivalent Circuits For Bridge Analysis.

"seen" by the load, Z_{eq1}. Using the delta-wye transformation for simplification, Z_{eq1} is computed to be,

$$Z_{eq1} = \frac{Z_b Z_c}{Z_b+Z_c+Z_d} + \frac{\left(\frac{Z_b Z_d}{Z_b+Z_c+Z_d} + Z_a\right)\left(\frac{Z_c Z_d}{Z_b+Z_c+Z_d} + Z_p\right)}{\frac{Z_b Z_d}{Z_b+Z_c+Z_d} + Z_a+Z_p+\frac{Z_c Z_d}{Z_b+Z_c+Z_d}} \qquad (43a)$$

From Chapter III, the values used for the bridge arm impedances to balance a typical value of sample and probe impedance are:

Bridge I

$$Z_b = Z_1 = 1.37K + j\,0.0 = 1.37K \angle 0^\circ \text{ ohms}$$

$$Z_c = Z_2 = 410 + j\,0.0 = 410 \angle 0^\circ \text{ ohms}$$

$$Z_a = Z_s = \frac{1.00\,K}{1+j(2.54\times10^{-4})} \approx 1.00\,K \angle 0^\circ \text{ ohms}$$

$$Z_p = Z_p = \frac{300}{1+j(6.02\times10^{-5})} \approx 300 \angle 0^\circ \text{ ohms}$$

$$Z_d = Z_d = 1.12\,m + j0.0 = 1.12\,m \angle 0^\circ \text{ ohms}$$

Bridge II

$$Z_b = Z_s = \frac{1.00\,K}{1+j(3.61\times10^{-4})} \approx 1.00\,K \angle 0^\circ \text{ ohms}$$

$$Z_c = Z_1 = 0.0 - j\,801 = 801 \angle -90^\circ \text{ ohms}$$

$$Z_a = Z_2 = 375 + j0.0 = 375 \angle 0^\circ \text{ ohms}$$

$$Z_p = Z_p' = \frac{300}{1+j(6.02\times10^{-5})} \approx 300 \angle 0^\circ \text{ ohms}$$

$$Z_d = Z_d' = 1.12\text{m} + j0.0 = 1.12 \text{ m} \angle 0^\circ \text{ ohms}$$

Using the numerical values for Z_a, Z_b, Z_c, and Z_p given above, then:

Bridge I

$$|Z_{eq1}| = 546 \text{ ohms} \tag{43a}$$

Bridge II

$$|Z_{eq1}| = 725 \text{ ohms} \tag{44a}$$

Thus the magnitude of Z_{eq1} is more than two orders of magnitude greater than the magnitude of Z_g for both bridge configurations. Therefore, the assumption of $Z_g \to 0$ is reasonable.

To show that the second ideal assumption is closely approximated, namely $Z_d \approx \infty$, it must be shown that $Z_d \gg Z_{eq2}$ where Z_d is the input impedance of the detector and Z_{eq2} is the effective impedance between the bridge arms as shown in Figure A3. Since $Z_g = 5.4$ ohms is two orders of magnitude less than $Z_c + Z_p$ and $Z_b + Z_a$ for both Bridge I and Bridge II, it is neglected in the computation of Z_{eq2}:

$$Z_{eq2} = \frac{Z_b Z_a}{Z_b+Z_a} + \frac{Z_p Z_c}{Z_p+Z_c} \tag{45a}$$

Inserting the typical impedance arm values into the expression for Z_{eq2},

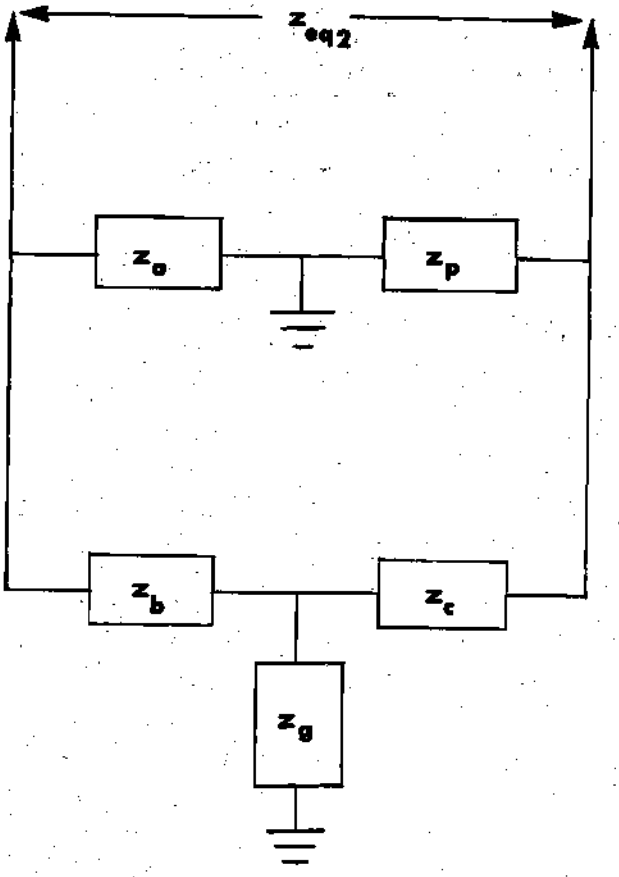


Figure A3. Equivalent Circuit For Bridge Analysis.

Bridge I

$$|Z_{eq2}| = 754 \text{ ohms} \tag{46a}$$

Bridge II

$$|Z_{eq2}| = 496 \text{ ohms} \tag{47a}$$

The input impedance of the detector is essentially the bias resistors on the gates of the two FET buffer stages shown in Figure 4, page 13, which are 3.3 and 1.0 meg-ohms each. Since each of these bias resistors is more than three orders of magnitude greater than the impedances they shunt for both Bridge I and Bridge II, they appear as virtual open circuits compared to the magnitude of Z_{eq2}. Thus the assumption that $Z_d \rightarrow \infty$ is reasonable.

Under these ideal conditions Equation (40a) can be simplified. First, as $Z_g \rightarrow 0$,

$$K = \frac{Z_b(Z_c+Z_a)+Z_a(Z_b+Z_c+Z_d)}{Z_b(Z_c+Z_p)\left(Z_d+Z_a+Z_p+\frac{Z_dZ_a}{Z_b}\right)} \tag{48a}$$

Secondly, if $Z_d \rightarrow \infty$, Equation (48a) becomes:

$$K = \frac{Z_a}{(Z_b+Z_a)(Z_c+Z_p)} \tag{49a}$$

Using the typical values for Z_a, Z_b, Z_c, Z_p, and a typical value of ΔZ_p of $+5 \times 10^{-4}$ ohms calculated using $\overline{\Delta\rho}$ and $\overline{\Delta\epsilon}$ from Chapter IV, then,

Bridge I

$$|K\Delta Z_p| = 3.26 \times 10^{-7} << 1.00 \tag{50a}$$

Bridge II

$$|K\Delta Z_p| = 1.05 \times 10^{-7} << 1.00 \tag{51a}$$

Thus $K\Delta Z_p << 1$ for both bridges under the assumptions of $Z_g \to 0$ and $Z_d \to \infty$.

Since $Z_g \to 0$ and $Z_d \to \infty$ have been shown to be reasonable assumptions, then Equation (49a) for K is a valid assumption. Thus Equation (39a) becomes:

$$\Delta E_d = \frac{\Delta Z_p Z_d E}{\left(Z_p + Z_g + Z_c + \frac{Z_g Z_c}{Z_b}\right)\left(Z_d + Z_a + Z_p + \frac{Z_d Z_a}{Z_b}\right)} \tag{52a}$$

As $Z_g \to 0$, ΔE_d becomes,

$$\Delta E_d = \frac{E\Delta Z_p Z_d}{(Z_p + Z_c)\left(Z_d + Z_a + Z_p + \frac{Z_d Z_a}{Z_b}\right)} \tag{53a}$$

Then as $Z_d \to \infty$, ΔE_d simplifies further,

$$\Delta E_d = \frac{E\Delta Z_p}{(Z_p + Z_c)(1 + Z_a/Z_b)} \tag{54a}$$

Rearranging Equation (54a) gives:

$$S = \frac{(\Delta E_d/E)}{(\Delta Z_p/Z_p)} = \frac{Z_b/Z_a}{(1 + Z_c/Z_p)(1 + Z_b/Z_a)} \tag{55a}$$

Using the typical numerical values for Z_p, Z_a, Z_b, and Z_c then:

Bridge I

$$S = .244 \tag{56a}$$

Bridge II

$$S = .198 \tag{57a}$$

The circuit diagram for the bridge detector unit is shown in Figure A4. Two FET common-drain amplifier stages are used for isolation to drive a differential amplifier. The two field effect transistors used in the buffer stages were not matched units. Potentiometer R_2 was inserted to balance out small differences in the characteristics of the transistors, and differences in the gain of each FET stage due to different loading effects, assuring that equal inputs to the buffer stages cause a minimum output at the detector.

The two common-drain FET stages can be analyzed using the equations developed in Millman and Halkias.[31] At 20 KHz the coupling capacitors C_1 and C_2 may be considered short circuits (their reactance is less than one ohm). Thus the voltage gain of the common-drain FET stage is given by:

$$A_v = \frac{g_m R_s}{1+R_s(g_m+g_d)} \tag{58a}$$

where

R_s = source resistance, in ohms

g_m = mutual conductance of the FET, in mhos

g_d = drain conductance of the FET, in mhos

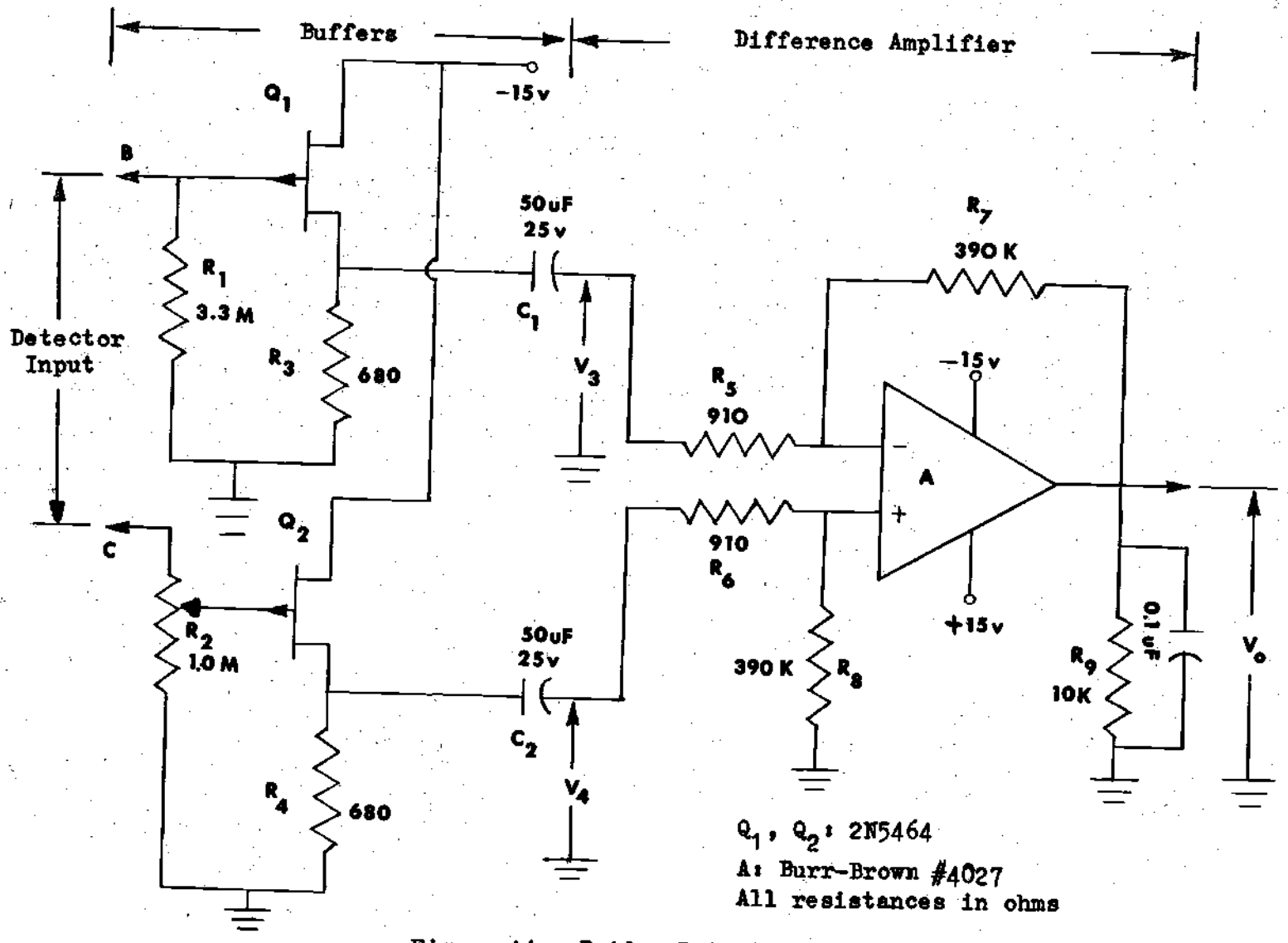


Figure A4. Bridge Detector Schematic.

Typical values for the 2N5464 FET's are $g_m = 3000\ \mu$mhos, and $g_d = 50$ μmhos. From Figure A4, for Q_1, $R_s = R_3 || R_5 = 390$ ohms. For Q_2, $R_s = R_4 || (R_6 + R_3) = 680$ ohms. Thus, using these values of R_s in Equation (58a) then:

$$A_{v1} = .536 \tag{59a}$$

$$A_{v2} = .663 \tag{59b}$$

where $A_{v1}(A_{v2})$ is the voltage gain of the common-drain FET stage containing $Q_1(Q_2)$. Potentiometer R_2 is used as an attenuator, to equate the two voltage gains. Thus, the lower value of A_{v1} will be used for further analysis.

The function of the differential amplifier is to amplify the difference between the two signals V_3 and V_4. The output signal of a practical differential amplifier is a function of both the difference signal, V_d of the two inputs, and the common mode signal, V_c of the two inputs. V_d and V_c are defined as,

$$V_d = V_4 - V_3 \tag{60a}$$

$$V_c = \tfrac{1}{2}(V_3 + V_4) \tag{61a}$$

The output voltage, V_o, depends upon the difference-mode voltage and common-mode voltage gains multiplied by V_c and V_d according to:

$$V_o = A_d V_d + A_c V_c \tag{62a}$$

From Figure A4, (assuming ideal operational amplifier parameters),

$$V_o|_{V_{4=0}} = -\left(\frac{R_7}{R_5}\right) V_3 \tag{63a}$$

$$V_o|_{V_{3=0}} = \frac{R_5+R_7)}{R_5} \cdot \frac{R_8}{(R_6+R_8} V_4 \tag{64a}$$

Therefore, by superposition,

$$A_c = \frac{-R_7}{R_5} + \frac{R_8(R_5+R_7)}{R_5(R_6+R_8)} \tag{65a}$$

$$A_c = \frac{R_8R_5-R_7R_6}{R_5(R_6+R_8)}$$

For an ideal difference amplifier, $A_c = 0$. This occurs when,

$$R_8R_5 = R_7R_6 \tag{66a}$$

The difference-mode gain A_d, then simplifies to:

$$A_d = \frac{R_8(R_5+R_7)}{R_5(R_6+R_8)} \tag{67a}$$

Using the values from Figure A4,

$$A_d = 428 \tag{68a}$$

The composite buffer-difference amplifier voltage gain is the product of A_v and A_d, or:

$$G_d = A_{v1} A_d \tag{69a}$$

$$G_d = .536 \times 428 = 229 \tag{70a}$$

Rearranging Equation (55a) to solve for $\Delta Z_p / Z_p$ gives,

$$\frac{\Delta Z_p}{Z_p} = \left(\frac{\Delta E_d}{E}\right) \times \left(\frac{1}{S}\right) \tag{71a}$$

The Hewlett-Packard model 120B oscilloscope can easily detect a 5 mV voltage signal. Therefore the maximum undetectable signal which could exist across the input to the detector is:

$$\Delta E_d(\max) = \frac{5MV}{229} = .022 \quad MV \tag{72a}$$

For the typical values of impedance used in Bridge I and II, the maximum value of ΔE_d, and the values of S, Equations (56a) and (57a), then Equation (71a) becomes,

Bridge I

$$\frac{\Delta Z_p}{Z_p} = \left(\frac{.022}{100}\right) \times \left(\frac{1}{.244}\right) = .00090 \Rightarrow .090\% \tag{73a}$$

Bridge II

$$\frac{\Delta Z_p}{Z_p} = \left(\frac{.022}{100}\right) \times \left(\frac{1}{.198}\right) = .00111 \Rightarrow .111\% \tag{74a}$$

Thus, since ΔZ_p can be either positive or negative, the theoretical errors due to the sensitivity of both Bridge I and Bridge II are less than $\pm$ 0.2 per cent each.

Readout Errors

Bridge I was used to find the blood plasma-$CaCl_2$ and probe resistance R_p. From Equation (18a), $R_p = R_2R_s/R_1$ where R_2/R_1 is a constant. Bridge II was used to measure the blood plasma-$CaCl_2$ and probe capacitance C_p. From Equation (33a), $C_p = C_1R_s/R_2$ where C_1/R_2 is a constant. The resistance standard, R_s, used for both the measurement of the blood plasma resistance and capacitance, is a 2 K ohm, ten turn, helipot (linearity $\pm$ 0.2 per cent) with vernier drive readable to three significant figures. Therefore, the readability is $\pm$ 1 unit out of 1000 or $\pm$ 0.1 per cent, and the nonlinearity of the helipot contributes $\pm$ 0.2 per cent error.

Summary

It has been shown for both Bridge I and Bridge II, that errors in bridge sensitivity are less than $\pm$ 0.2 per cent, readout errors contribute $\pm$ 0.1 per cent error, and nonlinearity errors for each bridge are $\pm$ 0.2 per cent. Therefore, bounds can be set on the total impedance measurement error:

Resistive Error = Readout Error + Sensitivity Error + Nonlinearity Error =

$\pm$ 0.1 per cent + $\pm$ 0.2 per cent + $\pm$ 0.2 per cent =

$\pm$ 0.5 per cent

Reactive Error = Readout Error + Sensitivity Error + Nonlinearity Error =

$\pm$ 0.1 per cent + $\pm$ 0.2 per cent + $\pm$ 0.2 per cent =

$\pm$ 0.5 per cent

The bridge system will detect Z_p changes as small as $\pm$ 0.5 per cent. Thus, impedance changes of one per cent are well within the accuracy limitations of the measuring system.

APPENDIX C

REDUCED EXPERIMENTAL DATA ON THE IMPEDANCE VARIATIONS OF HUMAN BLOOD PLASMA DURING COAGULATION

	Before Coagulation		After Coagulation		Resistivity Change	Permittivity Change
Test	ρ_i (ohm-cm.)	ϵ_i (pF/cm.)	ρ_f (ohm-cm.)	ϵ_f (pF/cm.)	$\Delta\rho$ (ohm-cm.)	$\Delta\epsilon$ (pF/cm.)
1	67.82	6.34	68.83	6.42	1.01	.08
2	61.72	6.74	60.96	6.78	-.76	.04
3	96.77	7.17	97.28	7.21	.51	.04
4	79.50	5.63	78.49	5.87	-1.01	.24
5	53.85	5.75	54.36	6.15	.51	.40
6	76.45	5.95	75.69	6.38	-.76	.43
7	73.91	6.38	74.42	6.86	.51	.48
8	68.83	6.58	67.06	6.66	-1.77	.08
9	72.39	5.48	72.64	5.59	.25	.11
10	79.50	5.79	79.50	6.07	.00	.28
11	87.12	5.99	87.88	6.38	.76	.39
12	56.64	5.36	56.13	5.52	-.51	.16
13	77.98	7.05	78.49	7.25	.51	.20
14	99.57	5.44	98.81	5.87	-.76	.43
15	84.07	6.07	84.07	6.15	.00	.08

REDUCED DATA CONTINUED

Test	Before Coagulation		After Coagulation		Resistivity Change	Permittivity Change
	ρ_i	ϵ_i	ρ_f	ϵ_f	$\Delta\rho$	$\Delta\epsilon$
16	71.37	6.15	71.88	6.26	.51	.11
17	55.12	6.78	56.13	7.01	1.01	.23
18	71.37	6.66	71.12	6.86	-.25	.20
19	78.99	6.34	79.76	6.70	.77	.36
20	70.10	6.46	69.60	6.74	-.50	.28
21	72.90	6.78	73.41	7.01	.51	.23
22	94.23	6.42	93.47	6.50	-.76	.08
23	75.95	6.93	76.20	7.01	.25	.08
24	97.28	5.48	97.28	5.71	.00	.23
25	59.44	5.59	58.93	5.75	-.51	.16
26	53.85	5.36	53.50	5.87	-.35	.51
27	70.36	6.86	68.75	6.93	-1.41	.07
28	79.76	6.62	78.00	6.78	-1.76	.16
29	73.91	5.99	72.75	6.15	-1.16	.16
30	71.00	6.42	68.50	6.46	-2.50	.04
31	63.75	6.90	63.25	7.01	-.50	.11
32	70.61	6.86	69.00	7.05	-1.61	.19
33	93.75	5.48	93.25	5.52	-.50	.04
34	86.20	5.63	87.00	5.83	.80	.20

REDUCED DATA CONTINUED

	Before Coagulation		After Coagulation		Resistivity Change	Permittivity Change
Test	ρ_i	ϵ_i	ρ_f	ϵ_f	$\Delta\rho$	$\Delta\epsilon$
35	74.50	5.52	75.50	5.57	1.00	.23
36	77.75	5.95	77.25	6.38	-.50	.43
37	53.25	5.79	52.75	6.07	-.50	.28
38	96.75	5.87	96.25	6.15	-.50	.28
39	80.75	5.63	80.75	5.87	.00	.24
40	87.25	5.52	88.00	5.83	.75	.31
41	82.75	6.78	82.25	7.01	-.50	.23
42	54.25	7.01	54.50	7.33	.25	.32
43	70.75	6.62	70.75	6.86	.00	.24
44	90.25	6.03	89.75	6.26	-.50	.23
45	93.00	6.86	94.00	7.09	1.00	.23
46	54.75	6.66	55.00	6.74	.25	.08
47	54.25	5.44	53.75	5.59	-.50	.15
48	92.25	6.07	92.75	6.15	.50	.08
49	75.25	6.34	74.50	6.42	-.75	.08
50	91.25	5.44	90.75	5.83	-.50	.39
51	69.50	6.74	70.25	6.93	.75	.19
52	53.50	5.59	53.50	5.79	.00	.20
53	99.50	5.87	99.75	6.15	.25	.28

REDUCED DATA CONTINUED

	Before Coagulation		After Coagulation		Resistivity Change	Permittivity Change
Test	ρ_i	ϵ_i	ρ_f	ϵ_f	$\Delta\rho$	$\Delta\epsilon$
54	78.25	6.66	78.75	6.78	.50	.12
55	83.00	6.23	82.25	6.46	-.75	.23
56	57.75	7.01	57.50	7.09	-.25	.08
57	67.25	5.48	66.75	5.56	-.50	.08
58	62.75	5.59	63.50	5.63	.75	.04
59	70.50	6.46	70.00	6.54	-.50	.08
60	81.50	6.90	81.50	7.13	.00	.23
61	54.50	5.63	54.00	5.87	-.50	.24
62	62.25	6.66	65.75	7.01	.50	.35
63	54.25	6.86	53.50	6.93	-.75	.07
64	75.25	6.26	76.50	6.46	1.25	.20
65	96.00	6.03	95.00	6.34	-1.00	.31
66	86.75	5.95	86.75	6.23	.00	.28
67	67.00	6.42	68.50	6.46	1.50	.04
68	54.50	6.86	53.50	7.01	-1.00	.15
69	76.50	6.62	77.00	6.66	.50	.04
70	79.25	6.74	79.25	6.93	.00	.19
71	64.50	5.48	66.00	5.71	1.50	.23
72	74.75	6.07	74.25	6.38	-.50	.31

REDUCED DATA CONTINUED

	Before Coagulation		After Coagulation		Resistivity Change	Permittivity Change
Test	ρ_i	ϵ_i	ρ_f	ϵ_f	$\Delta\rho$	$\Delta\epsilon$
73	79.50	5.63	80.00	5.95	.50	.32
74	89.00	5.87	88.00	6.15	-1.00	.28
75	92.75	6.78	92.00	6.86	-.75	.08
76	90.25	5.83	92.25	5.99	2.00	.16
77	51.75	6.74	52.75	6.93	1.00	.19
78	97.25	6.93	95.50	7.13	-1.75	.20
79	95.25	6.07	95.75	6.34	.50	.27
80	84.25	6.23	84.50	6.38	.25	.15
81	67.75	6.11	68.75	6.26	1.00	.15
82	79.75	5.75	80.00	5.91	.25	.16
83	69.00	5.32	68.50	5.56	-.50	.24
84	70.25	6.34	69.00	6.62	-1.25	.28
85	75.00	6.78	77.00	6.97	2.00	.19
86	83.25	6.62	82.50	6.82	-.75	.20
87	55.25	5.48	54.25	5.71	-1.00	.23
88	51.00	5.71	51.00	5.79	.00	.08
89	77.75	6.38	77.00	6.50	-.75	.12
90	85.25	6.34	86.50	6.74	1.25	.40
91	78.50	6.86	79.25	6.93	.75	.07

REDUCED DATA CONTINUED

	Before Coagulation		After Coagulation		Resistivity Change	Permittivity Change
Test	ρ_i	ϵ_i	ρ_f	ϵ_f	$\Delta\rho$	$\Delta\epsilon$
92	83.00	7.01	81.50	7.21	-1.50	.20
93	65.75	5.87	65.25	6.15	-.50	.28
94	78.25	6.66	76.75	7.01	-1.50	.35
95	92.75	6.07	92.75	6.46	.00	.39
96	87.00	6.34	87.25	6.42	.25	.08
97	55.25	6.42	55.25	6.62	.00	.20
98	65.25	5.48	67.75	5.79	.50	.31
99	79.50	5.44	79.00	5.71	-.50	.27
100	53.25	5.79	52.25	5.95	-1.00	.16
101	76.75	5.40	76.75	5.95	.00	.55
102	55.75	5.83	56.50	5.87	.75	.04
103	69.75	5.59	70.75	5.79	1.00	.20
104	79.00	6.62	81.00	6.93	2.00	.31
105	95.75	6.46	95.25	6.62	-.50	.16
106	62.75	5.95	64.00	6.34	1.25	.39
107	62.00	6.78	61.50	6.86	-.50	.08
108	82.75	7.01	81.75	7.21	-1.00	.20
109	79.50	6.46	80.50	6.70	1.00	.24
110	82.25	5.48	82.25	5.79	.00	.31

REDUCED DATA CONTINUED

	Before Coagulation		After Coagulation		Resistivity Change	Permittivity Change
Test	ρ_i	ϵ_i	ρ_f	ϵ_f	$\Delta\rho$	$\Delta\epsilon$
111	92.75	6.23	92.25	6.42	-.50	.19
112	53.00	5.75	52.25	5.95	-.75	.20
113	60.25	5.67	59.00	5.79	-1.25	.12
114	64.75	6.78	65.25	7.01	.50	.23
115	79.25	6.42	78.25	6.50	-1.00	.08
116	81.25	6.66	81.50	6.86	.25	.20
117	60.75	6.93	60.00	7.01	-.75	.08
118	92.75	5.79	94.00	5.91	1.25	.12
119	53.25	5.87	54.00	6.03	.75	.16
120	75.50	5.44	75.50	5.56	.00	.12
121	99.75	6.03	97.75	6.15	-2.00	.12
122	94.00	5.83	94.75	5.99	.75	.16
123	85.25	5.75	83.75	5.91	-1.50	.16
124	70.75	5.56	69.75	5.99	-1.00	.43
125	62.75	6.19	63.25	6.46	.50	.27
126	65.50	7.01	67.00	7.21	-1.50	.20
127	88.25	5.40	87.25	5.56	-1.00	.16
128	93.25	5.36	94.00	5.52	.75	.16

REDUCED DATA CONTINUED

	Before Coagulation		After Coagulation		Resistivity Change	Permittivity Change
Test	ρ_i	ϵ_i	ρ_f	ϵ_f	$\Delta\rho$	$\Delta\epsilon$
129	58.50	5.95	59.50	6.30	1.00	.35
130	67.00	5.63	65.25	6.23	-1.75	.60
131	67.75	6.78	68.50	6.93	.75	.15
132	94.50	6.62	93.50	6.74	-1.00	.12
133	71.50	6.74	71.00	6.93	-.50	.19
134	85.75	6.93	85.00	7.05	-.75	.12
135	54.75	5.36	54.25	5.83	-.50	.47
136	56.50	5.83	57.25	6.15	.75	.32
137	70.25	6.42	70.25	6.50	.00	.08
138	67.00	6.26	65.75	6.46	-1.25	.20
139	68.25	6.93	67.50	7.09	-.75	.16
140	78.50	5.67	78.00	5.95	-.50	.28
141	72.25	6.15	73.25	6.38	1.00	.23
142	75.50	6.62	75.75	6.78	.25	.16
143	95.75	5.48	97.25	5.75	1.50	.27
144	82.75	5.95	85.00	6.03	2.25	.08
145	54.25	5.59	52.25	5.83	-2.00	.24
146	75.50	6.50	75.50	6.74	.00	.24
147	78.50	6.34	78.50	6.78	.00	.44

REDUCED DATA CONTINUED

	Before Coagulation		After Coagulation		Resistivity Change	Permittivity Change
Test	ρ_i	ϵ_i	ρ_f	ϵ_f	$\Delta\rho$	$\Delta\epsilon$
148	65.25	6.66	66.25	6.86	.00	.20
149	68.75	7.01	69.75	7.13	1.00	.12
150	85.25	5.87	86.50	6.07	1.25	.20
151	90.25	5.83	89.50	5.95	-.75	.12
152	84.00	6.38	85.00	6.46	1.00	.08
153	89.50	6.23	88.50	6.42	-1.00	.19
154	78.50	6.74	78.75	7.01	.25	.27
155	79.25	5.48	81.50	5.75	2.25	.27
156	74.50	5.83	76.75	6.03	2.25	.20
157	85.25	5.75	85.00	5.95	-.25	.20
158	91.75	6.42	90.25	6.46	-1.50	.04
159	50.75	6.46	50.75	6.62	.00	.16
160	60.25	6.86	61.25	6.93	1.00	.07
161	58.00	6.97	57.25	7.01	-.75	.04
162	76.50	6.62	75.25	6.93	-1.25	.31
163	98.25	5.44	96.75	5.87	-1.50	.43
164	74.50	6.07	74.75	6.15	.25	.08
165	79.00	6.38	78.75	6.58	-.25	.20
166	97.75	6.82	97.50	7.01	-.25	.19

REDUCED DATA CONTINUED

	Before Coagulation		After Coagulation		Resistivity Change	Permittivity Change
Test	ρ_i	ϵ_i	ρ_f	ϵ_f	$\Delta\rho$	$\Delta\epsilon$
167	97.25	5.83	99.00	5.95	1.75	.12
168	94.00	5.99	94.00	6.23	.00	.24
169	55.50	5.87	54.50	6.11	-1.00	.24
170	76.50	6.58	77.25	6.74	.75	.16
171	54.50	5.44	55.25	5.83	.75	.39
172	61.50	6.15	62.75	6.34	1.25	.19
173	67.75	5.79	69.00	6.30	1.25	.51
174	54.50	5.67	54.75	5.95	.25	.28
175	62.75	6.78	62.50	6.82	-.25	.04
176	79.00	5.83	81.00	6.03	2.00	.20
177	67.75	5.95	69.75	6.15	2.00	.20
178	69.00	5.67	67.50	5.87	-1.50	.20
179	57.75	6.58	57.50	6.74	-.25	.16
180	78.50	6.82	78.00	6.93	-.50	.11
181	92.00	5.44	90.25	5.59	.25	.15
182	83.00	5.87	83.50	6.03	.50	.16
183	70.25	6.11	70.25	6.26	.00	.15
184	68.75	6.34	69.75	6.62	1.00	.28
185	79.50	6.74	81.00	6.82	1.50	.08

REDUCED DATA CONTINUED

	Before Coagulation		After Coagulation		Resistivity Change	Permittivity Change
Test	ρ_i	ϵ_i	ρ_f	ϵ_f	$\Delta\rho$	$\Delta\epsilon$
186	73.25	5.67	74.00	5.99	.75	.12
187	85.75	6.03	85.25	6.15	-.50	.12
188	86.50	5.44	87.25	5.79	.75	.35
189	87.75	6.54	87.50	6.70	-.25	.16
190	70.25	6.74	70.00	6.82	-.25	.08
191	71.00	6.62	71.50	6.78	.50	.16
192	52.00	6.34	51.75	6.54	-.25	.20
193	55.75	6.78	56.50	6.86	.75	.08
194	66.75	5.95	67.00	5.99	.25	.04
195	82.25	7.01	83.50	7.21	1.25	.20
196	75.25	5.79	75.25	5.87	.00	.08
197	70.75	6.42	71.50	6.50	.75	.08
198	72.75	5.56	75.25	5.87	2.50	.31
199	92.75	5.99	92.00	6.19	-.75	.20
200	91.00	5.75	91.00	5.95	.00	.20
201	84.75	5.59	82.50	5.83	-2.25	.24
202	90.00	6.74	90.25	6.90	.25	.16
203	71.00	5.44	71.00	5.56	.00	.12

REDUCED DATA CONTINUED

	Before Coagulation		After Coagulation		Resistivity Change	Permittivity Change
Test	ρ_i	ϵ_i	ρ_f	ϵ_f	$\Delta\rho$	$\Delta\epsilon$
204	78.50	6.34	76.75	6.50	-1.75	.16
205	90.75	6.07	90.00	6.46	-.75	.39
206	81.00	6.62	80.25	6.74	-.75	.12
207	92.00	5.48	92.00	5.71	.00	.23
208	67.25	5.99	68.50	6.23	1.25	.24
209	63.25	5.79	62.00	5.95	-1.25	.16
210	65.75	7.01	65.00	7.25	-.75	.24
211	97.75	6.15	99.25	6.30	1.50	.15
212	99.00	5.56	97.50	5.83	-1.50	.27
213	85.25	6.07	85.75	6.23	.50	.16
214	90.50	6.38	90.00	6.54	-.50	.16
215	82.75	6.19	84.75	6.30	2.00	.11
216	74.50	6.34	77.25	6.58	2.75	.24
217	85.50	6.86	87.50	7.01	2.00	.15
218	65.25	5.87	63.50	6.07	-1.75	.20
219	59.50	6.11	57.75	6.34	-1.75	.23
220	60.25	6.34	59.50	6.42	-.75	.08
221	71.50	5.99	72.75	6.23	1.25	.24
222	59.25	7.01	60.25	7.17	1.00	.16

REDUCED DATA CONTINUED

	Before Coagulation		After Coagulation		Resistivity Change	Permittivity Change
Test	ρ_i	ϵ_i	ρ_f	ϵ_f	$\Delta\rho$	$\Delta\epsilon$
223	62.75	6.74	61.50	6.93	-1.25	.19
224	76.75	5.87	77.75	5.95	1.00	.08
225	90.50	5.99	92.00	6.11	1.50	.12
226	57.75	5.44	58.75	5.75	1.00	.31
227	86.75	5.79	85.00	6.07	-1.75	.28
228	79.50	5.56	79.75	5.79	.25	.23
229	92.00	6.78	92.50	7.01	.50	.23
230	53.25	5.83	52.50	6.07	-.75	.24
231	74.75	6.07	72.75	6.15	-1.50	.08
232	75.50	6.54	75.25	6.70	-.25	.16
233	56.50	6.19	57.50	6.54	1.00	.35
234	62.25	5.95	62.50	6.23	.25	.28
235	67.00	6.58	67.00	6.66	.00	.08
236	78.50	6.78	79.00	6.90	.50	.12
237	81.75	5.83	81.75	6.03	.00	.20
238	79.75	6.07	80.00	6.23	.25	.16
239	93.25	5.99	94.50	6.26	1.25	.27
240	63.50	6.62	62.50	6.86	-1.00	.24
241	54.50	5.48	54.00	5.67	-.50	.19

REDUCED DATA CONTINUED

	Before Coagulation		After Coagulation		Resistivity Change	Permittivity Change
Test	ρ_i	ϵ_i	ρ_f	ϵ_f	$\Delta\rho$	$\Delta\epsilon$
242	59.25	6.78	57.50	7.09	-1.75	.31
243	69.50	6.23	70.25	6.38	.75	.15
244	75.25	5.71	75.25	5.87	.00	.16
245	76.50	5.56	76.25	5.63	-.25	.07
246	72.25	6.26	73.75	6.58	1.50	.32
247	88.00	5.99	87.50	6.23	-.50	.24
248	76.75	6.74	76.75	6.78	.00	.04
249	64.25	6.62	64.00	6.86	-.25	.24
250	97.50	6.07	97.75	6.30	.25	.23
251	69.50	5.48	69.50	5.67	.00	.19
252	59.00	5.71	60.00	5.87	1.00	.16
253	53.50	5.56	53.00	5.63	-.50	.07
254	79.00	6.62	77.25	6.82	-1.75	.20
255	71.75	6.58	70.00	6.24	-1.75	.16
256	75.50	6.50	78.00	6.78	2.50	.28
257	97.75	6.54	97.75	6.70	.00	.16
258	84.00	6.74	85.00	7.01	1.00	.27
259	78.50	6.34	77.25	6.46	-1.25	.12

REDUCED DATA CONTINUED

	Before Coagulation		After Coagulation		Resistivity Change	Permittivity Change
Test	ρ_i	ϵ_i	ρ_f	ϵ_f	$\Delta\rho$	$\Delta\epsilon$
260	68.25	5.83	69.00	6.03	.75	.20
261	67.00	5.63	67.50	5.75	.50	.12
262	87.00	5.59	85.50	5.71	-1.50	.12
263	72.75	6.19	72.50	6.46	-.25	.27
264	76.75	6.62	77.25	6.78	.50	.16
265	97.50	6.74	96.75	6.93	-.75	.19
266	85.50	5.48	87.00	5.71	1.50	.23
267	82.75	5.36	82.75	5.52	.00	.16
268	72.25	5.44	72.50	5.79	.25	.35
269	56.75	6.34	57.75	6.58	1.00	.24
270	54.00	5.71	56.00	5.95	2.00	.24
271	79.75	5.83	77.00	6.03	-2.75	.20
272	75.75	7.01	75.25	7.17	-.50	.16
273	60.50	6.82	60.25	6.93	-.25	.11
274	67.75	5.63	69.00	5.95	1.25	.32
275	77.00	5.75	77.00	5.91	.00	.16
276	78.25	6.78	77.25	6.86	-1.00	.08
277	81.75	5.44	82.00	5.83	.25	.39
278	80.00	5.63	80.00	5.95	.00	.32

REDUCED DATA CONTINUED

	Before Coagulation		After Coagulation		Resistivity Change	Permittivity Change
Test	ρ_i	ϵ_i	ρ_f	ϵ_f	$\Delta\rho$	$\Delta\epsilon$
279	54.00	5.79	56.00	5.87	2.00	.08
280	74.50	5.99	76.75	6.15	2.25	.16
281	55.75	5.87	55.25	5.99	-.50	.12
282	57.75	6.93	57.25	7.01	-.50	.08
283	66.75	5.59	67.75	5.75	1.00	.16
284	63.25	6.03	63.25	6.23	.00	.20
285	79.25	6.15	78.00	6.26	-1.25	.11
286	76.00	6.42	77.00	6.50	1.00	.08
287	74.75	6.74	76.75	6.93	2.00	.19
288	84.00	5.44	82.75	5.87	-1.25	.43
289	92.75	6.78	94.00	6.86	1.25	.08
290	89.50	6.23	90.00	6.42	.50	.19
291	67.25]	6.30	67.00	6.58	-.25	.28
292	78.50	6.74	78.50	6.93	.00	.19
293	81.75	5.63	82.50	5.95	.75	.32
294	70.75	5.56	70.25	5.87	-.50	.31
295	71.50	6.26	73.50	6.46	2.00	.20
296	86.75	6.34	86.75	6.58	.00	.24
297	98.50	6.07	98.75	6.23	.25	.16

REDUCED DATA CONTINUED

	Before Coagulation		After Coagulation		Resistivity Change	Permittivity Change
Test	ρ_i	ϵ_i	ρ_f	ϵ_f	$\Delta\rho$	$\Delta\epsilon$
298	91.75	6.07	98.75	6.23	.25	.16
299	57.00	7.01	59.00	7.17	2.00	.16
300	75.50	6.42	74.25	6.62	-1.25	.20
					$\Sigma\Delta\rho = 12.14$	$\Sigma\Delta\epsilon = 60.09$

APPENDIX D

COMPUTER PROGRAM FOR COMPUTATION OF CORRELATION COEFFICIENT

The following computer program was written in Fortran IV language for the Univac 1108 digital computer, to compute $r_{\Delta\rho\Delta\epsilon}$ the correlation coefficient of the resistivity and permittivity changes for the 300 samples tested.

```
1       DIMENSION X(300,2), COR(2,2,200), TITLE(12), IN(2),
2      1 XBR (2,200), XBRL(2,200), S(2,200), SL(2,200)
3     20 READ(5,1,END=8) TITLE
4        READ(5,2) N, NSRS, M, IT
5      1 FORMAT(12A6)
6      2 FORMAT(4I5)
7        DO 25 I=1, NSRS
8     25 READ(5,3) (X(J,I), J=1, N)
9      3 FORMAT(12F6.0)
10       DO 22 I=1, NSRS
11    22 IN(I)=I-1
12       CALL AUXCOR(X, N, NSRS, M, IT, COR, XBR, XBRL, S, SL, 300, 2, 200)
13       WRITE (6,4) TITLE
14     4 FORMAT(1H1 12A6/47HOMATRICES OF AUTO AND CROSS CORRELATIONS
```

APPENDIX D (CONTINUED)

```
      (I,J,L)1)
      DO 30 L=1, M
      L1=L-1
      WRITE(6,5) L1
    5 FORMAT(30X,4HLAG=I3)
      WRITE(6,6) (IN(K),K=1,NSRS)
    6 FORMAT(3H J=217)
      DO 30 I-1, NSRS
   30 WRITE(6,7) IN(I), (COR(I,J,L),J=1, NSRS)
    7 FORMAT(3H I=14,2F7.4)
      GO TO 20
    8 STOP
      END
```

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